TITLE THE EFFECTS OF OESTROGEN ON RENAL AND SYSTEMIC HAEMODYNAMICS IN THE RAT: INFLUENCE OF INTRARENAL VASOACTIVE SUBSTANCES AND PLASMA VOLUME STATUS

AUTHOR John Kenrick Evans

INSTITUTION and DATE University of Keele 1986

THE EFFECTS OF OESTROGEN ON RENAL AND SYSTEMIC HAEMODYNAMICS IN THE RAT: INFLUENCE OF INTRARENAL VASOACTIVE SUBSTANCES AND PLASMA VOLUME STATUS

by

John Kenrick Evans

A thesis submitted to the University of Keele in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

University Department of Postgraduate Medicine, North Staffordshire Medical Institute Research Laboratories.

October 1986

CONTENTS

List of Pages Containing Figures and Tables

Figures

Acknowledgements and Statement of Collaboration

I am indebted to Bristol-Meyers Pharmaceuticals and the North Staffordshire Medical Institute, whose financial assistance made this investigation possible.

I wish to express my sincere thanks to Professor G. M. Aber and Doctor P. F. Naish for the support and invaluable advice given to me during the course of this investigation, for which I am extremely grateful. My gratitude also to Dr. M. Collins for statistical advice, and Mr G. Darlington for his assistance during the majority of the animal experiments described in this thesis.

I would like to acknowledge the contribution of Dr. E. M. F. McCale to this investigation. I am grateful to Dr McCale with regard to the determination of plasma renin substrate concentration and quality control data - both in connection with the radioimmunoassays described in this thesis.

Finally, to Carys, for the unselfish manner in which you have prepared this document - gramercy!

Published Work Contained in this Thesis

1. Evans, J.K, Smith, R.S, McCale, E.M.F, Naish, P.F. and Aber, G.M. (1984). The Effect of Oestrogen on Renal Blood Flow. Clinical and Experimental Hypertension. B,3, 444.

2. Evans, J.K, Bennett, J, Smith, R.S, Naish, P.F. and Aber G.M. Interrelationships between oestrogen-induced changes in renal haemodynamics and the renin-angiotensin system. Kidney International (in press).

3. Evans, J.K, Naish, P.F. and Aber, G.M.(1986). Oestrogen-induced changes in renal haemodynamics: influence of plasma and intrarenal renin. Clinical Science , 71, 613-619.

ABSTRACT

The effects of oestrogen on renal and systemic haemodynamics, intrarenal vasoactive substances and plasma volume status have been studied in adult female normotensive and spontaneously hypertensive rats.

Administration of oestrogen to both strains of rat resulted in a reduction in renal blood flow, due to an increased renal vascular resistance in the presence of an increase in both plasma renin substrate concentration and activity.

In the normotensive rat, oestrogen affected a rise in arterial pressure, accompanied by an expanded plasma volume, and either an unchanged or increased plasma renin activity. Whilst changes in arterial pressure were reciprocally related to changes in plasma volume, no relationship was noted between arterial pressure and plasma renin activity. In the spontaneously hypertensive rat, oestrogen caused a fall in arterial pressure, due to a reduced peripheral vascular resistance.

The oestrogen-induced reduction in renal perfusion was due to enhanced local, intrarenal generation of angiotensin II - as evidenced by an increase in renal perfusion in both strains of oestrogen treated rat after angiotensin converting enzyme inhibition (captopril). Evidence from studies involving prostaglandin synthesis inhibition (indomethacin), plasma prorenin activation (trypsin) and saralasin treatment (angiotensin II blockade)indicate that a proportion of this angiotensin II-mediated intrarenal vasoconstriction involved at least a prostaglandin-dependant pathway, in addition to an alteration in intrarenal vascular angiotensin II receptor density.

The oestrogen-induced rise in arterial pressure was determined principally by plasma volume expansion, although angiotensin II also contributed to this rise - as demonstrated by a lessening of oestrogen-induced hypertension by captopril. The oestrogen-induced fall in arterial pressure appeared to be prostaglandin-dependant, although indomethacin failed to influence arterial pressure in oestrogen treated spontaneously hypertensive rats.

This investigation demonstrates that an alteration in the normal equilibrium between intrarenal vasoactive substances can, in conjunction with a change in plasma volume status, lead to disturbances in both renal and systemic haemodynamics during oestrogen treatment.

Chapter 1

GENERAL INTRODUCTION

1.1 THE PHYSIOLOGICAL ROLE OF THE RENAL CIRCULATION

In the kidney, as in the lung, only a relatively small proportion of the large fraction of cardiac output flowing through the organ (approximately 20%) is concerned with the nutrition of the tissues themselves. The greater part of this blood has to pass through a complicated intrarenal pathway so that its pH, osmolality and composition may be regulated. The anatomy and physiology of the renal circulation are thus of the greatest importance not only to renal function itself, but also to extrarenal processes, principally the maintenance of overall cardiovascular homeostasis. Thus, a low medullary perfusion rate, especially in the papilla, is critical for the efficient function of the countercurrent system, as this prevents the continuous washout of the osmolal gradient created by tubular function - thereby permitting the production of a hypertonic urine. Likewise, transplantation of a healthy kidney often normalises blood pressure in patients with severe renal disease and accompanying hypertension.

From the above it is clear that precise control of the renal circulation is central to normal renal physiology, and that the factors which both mediate and influence this control have the potential to affect the regulation of both renal and systemic haemodynamics. A detailed description of the renal circulation may be found in Fourman and Moffat (1). The organisation of the principal renal blood vessels, and their juxtaposition to the functional unit of the kidney, the nephron, is illustrated in Figures 1.1 - 1.3.

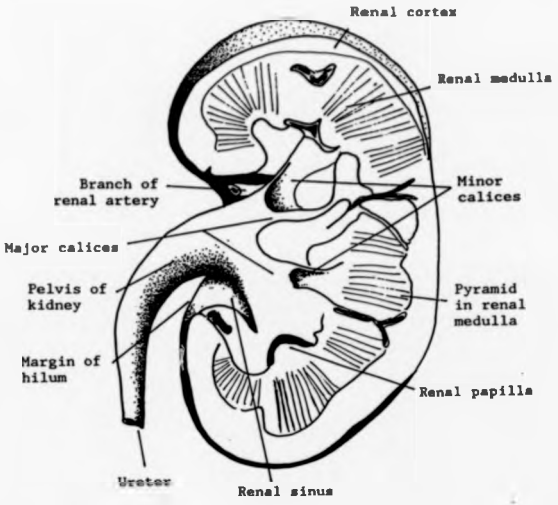


Figure 1.1 illustrates the structural organisation of the mammalian kidney by means of a longitudinal section through a human kidney.

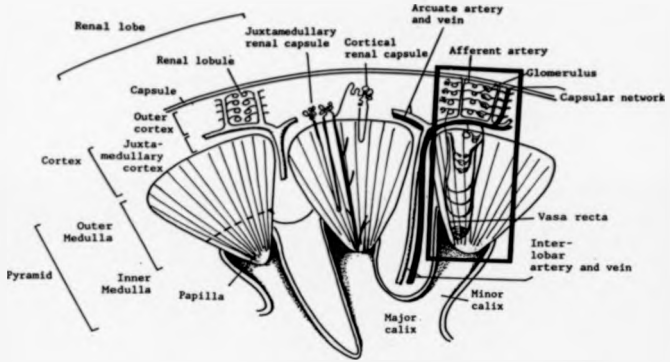


Figure 1.2 depicts the basic arrangement of the principal blood vessels in the mammalian kidney.

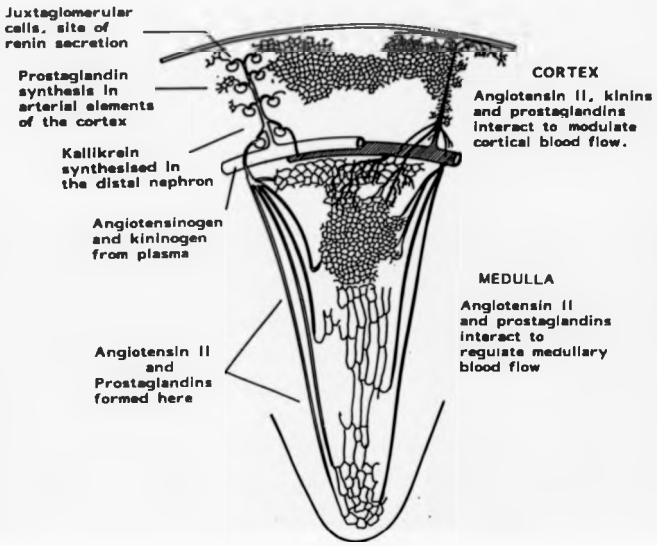


Figure 1.3 Schematic representation denoting potential roles of the renin - angiotensin and kallikrein - kinin systems and renal prostaglandins as complementary modulators of the renal circulation (refer to text). Diagram is an expanded portion of Figure 1.2.

1.2 CONTROL OF THE RENAL CIRCULATION

Renal blood flow is subject to a type of control which seems to be more highly developed in the kidney than in other organs. As arterial pressure increases above 90 mm Hg, renal blood flow does not follow this increase in pressure, but stays almost constant. This so-called autoregulation of renal blood flow occurs within seconds, and has been found in innervated, denervated and isolated kidneys, and during blockade of intrarenal ganglia. The increase in vascular resistance which accompanies an increase in perfusion pressure appears to be a consequence of wholly intrarenal mechanisms.

It need be emphasised, that although renal perfusion is ordinarily little influenced by perfusion pressure, this does not mean that intrarenal perfusion is not influenced by a number of local factors (described below). It is indeed so influenced under a variety of circumstances that the renal vascular response probably plays a pivotal role as a determinant of renal function.

Many hypotheses have been elaborated in an attempt to account for autoregulation. These hypotheses include consideration of the effects of perfusion pressure and a number of endogenous vasoactive factors, including the renin-angiotensin and kallikrein-kinin systems, prostaglandins, noradrenaline and the sympathetic nervous system. No one mechanism has been shown to fully account for autoregulation, which is undoubtedly under multifactorial control. However, two main theories, the myogenic and vasoactive theories, have gained widespread acceptance. According to the myogenic hypothesis the stimulus for the vascular smooth muscle contraction in

response to increasing intraluminal pressure is either the transmural pressure itself, or the increase in tangential tension of the vascular wall (2,3). According to this hypothesis the vascular smooth muscle is pressure or tension sensitive. The vasoactive hypothesis states that local vasoactive substances such as angiotensin II, prostaglandins or kinins are released in response to changes in perfusion pressure, appropriately changing local vascular resistance (4,5). Whereas the weight of evidence available to date is consistent with the myogenic hypothesis, the involvement of local intrarenal vasoactive substances in the control of renal blood flow is less clearly defined.

Renin, a proteolytic enzyme, is produced primarily in the renal cortex, with much smaller amounts being found in the uterus, placenta, salivary glands, brain and some blood vessels. In the kidney renin is synthesised in specialised cells, juxtaglomerular cells, of the renal afferent arteriole, in intimate contact with the distal tubule (134,203,204). The juxtaglomerular cells are thought to be modified smooth muscle cells (205). The macula densa, intimately associated with the juxtaglomerular cells, consists of an elongated group of columnar epithelial cells situated in the distal convoluted tubule as it passes the glomerulus after it ascends from the medulla (205). Collectively, the juxtaglomerular cells, macula densa and afferent glomerular arteriole are termed the juxtaglomerular apparatus.

Due to its unique structure and location (adjacent to the vascular pole of the glomerulus), early investigators postulated a role for the juxtaglomerular apparatus in the modulation of renal function. It was proposed that the

structure released an agent that could form or activate a vasoactive substance which, in turn, influenced local vascular tone (206). The major area of interest in this field was, however, oriented toward non-renal functions such as in the control of aldosterone release, the regulation of systemic cardiovascular function and the pathophysiology of hypertension (207). More recent investigations have, nevertheless, provided important evidence to support the contention of a physiological involvement of a locally generated pressor substance, angiotensin II, in the regulation of renal vascular resistance.

Renin secreted from the juxtaglomerular apparatus into the renal vascular segment acts on a plasma α-globulin. This angiotensinogen, synthesized by the liver, serves as a substrate to split off a precursor decapeptide, angiotensin I. Within the kidney angiotensin I does not exhibit any significant pressor activity, and a specific converting enzyme, produced primarily by the lung, splits two amino acids off the decapeptide to form the active moiety, angiotensin II. The majority of angiotensin II is thus generated in the pulmonary circulation, and may be found distributed throughout the systemic circulation.

Angiotensin II is the most active endogenous vasoconstrictor substance identified to date, with an especially marked influence on the renal vasculature (6). Numerous studies have identified the vascular target site for angiotensin II. The presence of these receptors has been demonstrated in vascular smooth muscle from several tissues (208-210), and it is generally accepted that smooth muscle cells of the renal vasculature possess similar receptors. In this context, recent studies have demonstrated the presence of angiotensin receptors on intraglomerular structure (211). Thus, cultured cells (of

mesangial origin) from human glomeruli have been shown to exhibit contractile activity (212), an effect which may be mediated by angiotensin II (213).

The findings from studies which have utilised intra-arterial infusions of angiotensin II are often cited as evidence supporting an involvement of the hormone in the regulation of renal vascular resistance. These studies have shown that intrarenal infusions of low, subpressor doses of angiotensin II, which do not alter peripheral circulating angiotensin II levels (214), consistently result in dose-dependant reductions in renal blood flow (215). This information suggests that the pressor effect of angiotensin II on the renal vasculature could be mediated by preformed angiotensin II, delivered to the kidney in arterial blood.

The role of the renin-angiotensin system has also been investigated by inhibition of the formation of angiotensin II, with either a converting enzyme inhibitor, or a peptide antagonist that inhibits the interaction of angiotensin II with its tissue receptors. Early studies used animals in which prior activation of the renin-angiotensin system had been achieved, by manoeuvres such as sodium depletion, renal artery occlusion, vena cava constriction or high output heart failure. Here, pharmacological interruption of the renin-angiotensin system resulted in a reduction in renal vascular resistance, with consequent large increases in renal blood flow (138,216,217). In most of these studies however, the changes noted in renal haemodynamics were associated with a reduction in mean arterial pressure.

More recently the infusion of angiotensin receptor antagonists intrarenally, at doses which only minimally affect blood pressure, has been achieved. As is the earlier studies this infusion also resulted in an increase

in renal blood flow in animals with a previously activated renin-angiotensin system (218,219). This observation suggests that aside from a systemic mode of action for angiotensin II, there is also a critical intrarenal focus for conversion of angiotensin I to angiotensin II. Powerful support for this thesis may be derived from reports which have demonstrated the presence of significant quantities of converting enzyme in renal tissue (220-222). Thus angiotensin II, generated intrarenally, may act as a local renal vascular hormone, appropriately modulating renal vascular tone (9-11).

Given the probability that angiotensin II is generated within the kidney and the hypersensitivity of the renal vessels to this substance, angiotensin II is ideally suited to the role of a local, physiological modulator of renal haemodynamics. Indeed, it would appear that renin release, with subsequent generation of angiotensin II, is the major determinant of renal perfusion pressure in situations where renal blood flow is compromised by either local factors such as lesions of the renal artery (12), or systemic factors such as diminished extracellular fluid volume (13). During sodium depletion for example, elevated renin-angiotensin system activity is a requisite for maintenance of systemic blood pressure and renal blood flow. Thus, whilst infusion of the angiotensin II blocking agent, 1-sar-8-ala angiotensin II, decreased blood pressure and glomerular filtration rate and increased renal blood flow in sodium-deplete dogs, it had no such effect in sodium-replete animals, in which the activity of the renin-angiotensin system is normal (13). Under conditions of high renin release by the kidney therefore, angiotensin II may act as a regulator of renal blood flow to maintain glomerular filtration rate (14).

The contribution of other intrarenal hormonal systems to the control of renal blood flow is poorly defined, as many components of these systems are not, as yet, adequately characterised, and many current assays employed to measure the activity of the systems are of uncertain specificity. Further, there is much inter-species variability in response to these hormones. However, there is a growing body of evidence to indicate that both the kallikrein-kinin system and renal prostaglandins form part of an important intrarenal hormonal regulatory complex which includes the renin-angiotensin system.

Prostaglandins are 20-carbon unsaturated carboxylic acids derived from essential fatty acids, such as arachidonic acid. Synthesis begins with the release of arachidonic acid from tissue stores, chiefly phospholipids, which is then acted upon by tissue cyclo-oxygenase to recruit the prostaglandin component in response to diverse stimuli. All renal arterial elements appear to be capable of prostaglandin synthesis, including the main renal artery and the lobar and interlobular arteries with attached afferent arterioles (a site favouring interactions with renin).

Early studies which demonstrated that prostaglandins could dilate many vascular beds, and in particular, the renal vascular bed, led to the idea that prostaglandins may be important modulators of renal blood flow (15). Whilst subsequent studies have shown that the intrarenal administration of either prostaglandins (16) or arachidonic acid (17) into the renal artery may cause a significant increase in renal blood flow, more physiological doses of prostaglandins appear to exert a much smaller effect on renal blood flow. However, good evidence supporting a role for renal prostaglandins as renal vasodilators under normal

conditions, come from studies in which pharmacological inhibitors of prostaglandin synthesis have been used. A number of studies have been performed which have shown that the administration of prostaglandin synthesis inhibitors, such as indomethacin and meclofenamate, resulted in a decrease in renal blood flow and an increase in renal vascular resistance (18,19).

Within the kidney kallikrein is predominantly located within the cortex, membrane bound in tubules and appears in urine fractions which originate from the distal nephron. The enzyme is probably synthesised in an inactive precursor form, which can be activated by trypsin and other enzymes. When released, renal kallikrein releases the decapeptide lysyl-bradykinin from a plasma alpha-globulin, kininogen. Kinins are amongst the most potent vasodilator agents known. Although it is clear that administration of kinins can alter renal blood flow (20) and sodium and water excretion (21), the role of kinins as physiological regulators of renal haemodynamics is less certain.

As stated earlier, the renin-angiotensin system, the kallikrein-kinin system and renal prostaglandins form an intrarenal hormonal regulatory complex which contributes to the regulation of the renal circulation. The enzymes renin and kallikrein are formed and stored intrarenally; when released they act on plasma globulins to liberate decapeptides, angiotensin I and lysyl-bradykinin respectively. A single enzyme acts on both decapeptides, converting angiotensin I to the active hormone angiotensin II, and degrading kinins. Blockade of this enzyme with an inhibitor will augment the activity of the kallikrein-kinin system (22) and depress that of the renin-angiotensin system (23). Although kinins and angiotensin II have

opposing effects on the renal circulation, they share an important property, the ability to promote prostaglandin synthesis and to alter the metabolism of prostaglandins (24). Further, release of renin and kallikrein is partially controlled by a prostaglandin mechanism(25,26). Thus, interactions between the renin-angiotensin and kallikrein-kinin systems and renal prostaglandins (Fig. 1.4) have the potential for major modifications of the effects of these hormonal systems on renal haemodynamics (Fig. 1.3). For example, vasodilators may be evoked to antagonise the vasoconstrictor action of angiotensin II, under conditions of high renin secretion, such as volume-depletion (27,28).

1.3 THE KIDNEY AND OVERALL CARDIOVASCULAR HOMEOSTASIS

The renal circulation is subject to strict, multifactorial control of renal origin, so as to maintain efficient renal function. A healthy renal circulation is also vital to extrarenal processes, namely cardiovascular homeostasis. This fact is confirmed by the central role the kidney plays in the regulation of blood pressure. Modern ideas of the involvement of the kidney in blood pressure control have evolved from workers such as Goldblatt, whose classic experiment of 1963 showed that renal ischaemia precipitated hypertension in dogs. More recently, the antihypertensive action of a normal kidney has been demonstrated by transplantation. For instance, there are two types of rat hypertension which show the same effect. In Dahl rats, for example, one can transplant a kidney from the DS strain (susceptible to hypertension) to a DR rat (resistant to hypertension) and the blood pressure of the DR rat will rise. One can also do the opposite, taking a kidney from a DR rat and transplanting it to a DS rat, and the blood pressure of the DS rat will drop. The same patterns of change have also been observed after transplantation in the Milan strain of

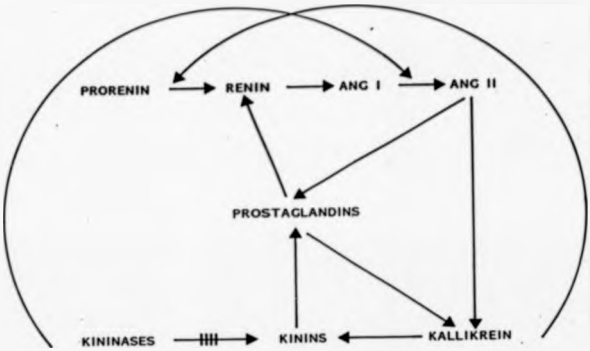


Figure 1.4. Interactions between the renin-angiotensin, kallikrein-kinin and prostaglandin systems. Solid arrows indicate a stimulatory pathway and the cross-hatched arrow an inhibitory pathway.

hypertensive rats. Similarly, when a person with end stage renal disease and severe hypertension receives a good kidney through transplantation, the blood pressure often returns to normal. Muirhead, (29) has reported that the renomedullary interstitial cells are the site of the antihypertensive action of the normal kidney.

These transplantation studies appear to demonstrate that the kidney can somehow carry the message of blood pressure and that the kidney can somehow command the level of blood pressure within the body. How might the kidney be able to achieve this?' The kidney is the source of a number of vasoactive compounds which are concerned with the control of renal blood flow. The fact that these compounds have also been strongly implemented in the regulation of blood pressure demonstrates the importance of the control of the renal circulation to blood pressure control, and vice versa. The systemic hormone angiotensin II, is one of the most potent pressor agents known. Angiotensin II acts to constrict arterioles directly, it causes the adrenergic nerves to secrete more noradrenaline, the adrenal medulla to secrete more adrenaline. It acts upon specific receptors within the brain to cause an increased sympathetic nerve outflow. It acts on the adrenal glomerulosa to cause more aldosterone action and it seems to act on renal tubules directly to cause sodium retention.

The vasodilatory properties of kinins and prostaglandins have been mentioned in relation to the control of the renal circulation. They may contribute to blood pressure regulation by maintaining the renal circulation in the face of pressor stimuli, contributing to the control of extracellular fluid volume and modulating the response of vascular smooth muscle to

pressor stimuli. The theory that these mechanisms operate in vivo is supported by studies which have shown firstly, low urinary kallikrein levels (an index of kallikrein secretion) in essential hypertensives (30), and secondly, that administration of prostaglandin synthesis inhibitors elevated blood pressure and systemic vascular resistance in normotensive individuals (31) and elevated blood pressure levels further in hypertensive humans (32).

In summary, the control of renal blood flow and blood pressure is mediated through a number of common intrarenal pathways involving vasoactive compounds. A disturbance in either of these variables may result in an adverse effect on the other variable, with untoward consequences for both normal renal function and overall cardiovascular homeostasis.

1.4 THE INFLUENCE OF OESTROGEN ON THE RENAL CIRCULATION

Oestrogen-containing compounds are widely accepted as a simple and effective form of oral contraception. There is however, an increasing awareness of their undesirable side effects, some of which may be serious, such as hypertension. In spite of these adverse effects few, if any, would advocate the abandonment of oral contraceptives. The advantages of family planning by this means, and the short term preferences of human nature are such that most women would probably accept an even greater risk to avoid an unwanted pregnancy, bearing in mind the risks of pregnancy itself, including hypertension and toxaemia. The fact is that oral contraceptives are probably here to stay. Although the relative risk in users is large, the absolute risk is small for women under the age of forty. Nevertheless, with an already substantial and still growing percentage of the female population taking oral contraceptives regularly (an

estimated 60 million worldwide in 1978), and likely to continue doing so for the rest of their reproductive lives, 'small risks' assume added significance.

Observations from a broad spectrum of studies involving the use of oestrogens suggest that these steroids may well have the capacity to exert a powerful influence on the renal circulation.

The first line of evidence stems from the effect of oestrogen on one of the major cardiovascular risk factors, blood pressure. There is now an overwhelming weight of evidence associating the use of oestrogen-containing oral contraceptives with the development of hypertension in certain susceptible women (33, 34). Practically all women ingesting oestrogen-containing oral contraceptives show a slight rise in blood pressure (35). Malignant hypertension is rare (36), but 5-15% of users can expect to become hypertensive. This prevalence is about twice that of women of the same age who are not taking an oral contraceptive. Many epidemiological studies utilising large populations have show that the height of systolic blood pressure is a potent predictor of subsequent cardiovascular disease (37). Further, actuarial studies indicate that the blood pressure most favouring longevity and low morbidity is the very lowest compatible with proper circulatory function. Observations of this type may explain the excess of deaths from non-rheumatic heart disease and cerebrovascular disease seen, particularly in older women ingesting oral contraceptives (38).

Secondly, oestrogens are known to influence elements of the intrarenal hormonal complex previously mentioned - particlarly the renin-angiotensin system. Oestrogens have been shown to

exert a variable effect on this system, the most consistant finding being an increase in plasma renin substrate concentration (39). Less consistant are the reports that oestrogens cause an increase in plasma renin activity (40). Thus the net effect of oestrogen on the renin-angiotensin system is stimulatory, resulting in increased levels of angiotensin II (41). The few reports available concerning oestrogens and the kallikrein-kinin system indicate that both kallikrein (42) and kininogen (43) concentrations may rise as a result of oestrogen treatment. Similarly, a number of studies have shown that oestrogen can stimulate the synthesis of prostaglandins from both vascular tissue (44, 45) and the kidney (46). Thus, as for the renin-angiotensin system, oestrogen may exert a stimulatory effect on both the kallikrein-kinin system and prostaglandin synthesis.

The third sphere of research to suggest that oestrogen may influence the renal circulation is that of smooth muscle receptor physiology. Oestrogens have been shown to alter smooth muscle sensitivity to a number of vasoactive compounds by modulating receptor physiology. For example, oestrogen can enhance the sensitivity of uterine tissue to angiotensin II (47), catecholamines (48) and Oxytocin (49), not via a greater affinity of uterine receptors for their respective hormones, but through an increase in the actual number of these receptors. The application of similar studies to the renal vasculature is at present beyond the current state of technical expertise. Recent work in our own laboratories, however, which has demonstrated that oestrogen can increase the number of angiotensin II receptors present in the glomeruli of normotensive rats (A.Messenger: personal com.)is in agreement with the 'uterine' studies.

Finally, a clinical, histological and angiographic study by Boyd et al(50), demonstrated the occurrence of impaired renal function, including renal failure, associated with intrarenal vascular lesions, in women taking an oestrogen-containing compound.

1.5 PURPOSE OF INVESTIGATION

Control of the renal circulation is critical to both normal renal function and overall cardiovascular homeostasis. Intrarenal vasoactive compounds are clearly involved in this control, although the precise nature of their involvement is not clear. A growing body of evidence now exists to suggest that oestrogens may exert a powerful influence on the renal circulation. Yet, in spite of this evidence, the influence of oestrogen on the renal circulation has received only minimal attention. Such studies that exist, lack the cohesive approach of taking haemodynamic measurements under controlled physiological conditions, in conjunction with measurements of relevant hormone and enzyme systems at tissue as well as at plasma levels.

The objective of this study therefore, was to study the influence of oestrogen on both renal and systemic haemodynamics, intrarenal vasoactive compounds, particularly angiotensin II, and plasma volume status. By adopting this multifaceted approach, any relationship between changes in renal and systemic haemodynamics may be elucidated, and equated to changes in both intrarenal vasoactive compounds and plasma volume.

Studies of this nature, involving invasive surgery and tissue measurements, are not possible in humans for ethical reasons. The most appropriate animal model must therefore be

used which in this case is generally held to be the pure-bred strains of normotensive and spontaneously hypertensive female rats. By studying both strains, it may be possible to firstly, achieve a better understanding of the impact of oestrogen on the renal circulation, and secondly, to identify differences in the circulatory and hormonal responses to oestrogen treatment between normotensive models and those animals with underlying 'essential hypertension'.

Chapter 2

MATERIALS AND METHODS

2.1 INTRODUCTION

The basic methods used throughout this investigation are described. These include a description of the application of radioactive microspheres and radioimmunoassay to measure variables of systemic and renal haemodynamics and the renin-angiotensin system.

2.2 ANIMALS

Adult female rats have been used throughout the study. Sprage-Dawley rats (Nottingham University) were used in the studies detailed in chapters 3-5&8. The spontaneously hypertensive strain of rat (SHR) and its normotensive Wistar counterpart (Olac Ltd, England) were used in the studies described in chapters 6,7. The experimental groups of animals used within each study have been age-matched.

Animals were housed at 21 ± 1°C, and unless otherwise stated, fed with special Diet Services (England) rat and mouse number 3 pellets (Sodium content = 0.34%, Potassium content = 0.74%) and allowed free access to tap water.

2.3 CONDITIONS OF STUDY

2.3.1 Oestrogen Administration

The natural oestrogen oestrone has been used throughout the study. Oestrone Acetate (Roussel Laboratories Ltd, England) dissolved in ethyl oleate (4 mg per ml) was administered by subcutaneous injection. Control animals received an appropriate injection of vehicle alone. Animals destined for either oestrogen or vehicle treatment were selected randomly, without bias. Likewise, animals were randomly selected into the various experimental protocols described in this investigation.

The doses of oestrone used during this investigation

were selected on the basis of the report of László, F.A. (51). This detailed study was designed, in part, to identify the dose of oestrone likely to influence renal vascular sensitivity to vasoactive compounds.

2.3.2 Anaesthesia

Haemodynamic measurements and tissue sampling as performed during this investigation involve invasive surgery and therefore, the use of an anaesthetic agent to induce a surgical plane of anaesthesia. The induction of anaesthesia however, has important implications regarding these procedures. Firstly, the use of an anaesthetic agent can result in cardiovascular instability from, for example, hypotension, brachycardia or a fall in cardiac output (52). In addition, the use of a barbiturate anaesthetic has been shown to cause a fall in renal blood flow, both in the rat (53) and dog (54). Secondly, induction of anaesthesia and induction of renin release are simultaneous events in the rat (55). Taking measurements from the calm,awake animal,post-operatively would preclude the occurrence of these undesirable events (56). The necessity of a long post-operative recovery period would, however, entail a prolonged period of discomfort for the animal, and would also introduce a real risk of infection. Further, radioactive microspheres are unsuitable for use in this scenario.

For the purpose of this investigation therefore, haemodynamic measurements and tissue samples were taken from anaesthetised animals. The inhalation agent, Methoxyfluorane (Penthrane, Abbott Labs. Ltd, England) was chosen, as this agent is claimed to exert a

minimal disturbance on both cardiovascular stability (manufacturer's claim) and renin release (55), and is suitable for the induction of a light plane of anaesthesia. It is clear however, that the results of this investigation must be interpretated in the light of the stressful and traumatic event, surgery, and in the context of a possible influence of anaesthesia on both renal and systemic haemodynamics and the renin-angiotensin system (see appendix).

2.4 VARIABLES MEASURED

2.4.1 Blood Pressure

Blood pressure may be measured by both direct and indirect methods in the rat. The direct method involves the invasive approach of arterial catheterisation - usually of the carotid artery. The implanted catheter is connected to a pressure transducer, and recordings are made using a polygraph (57). All indirect methods use an occlusive device and a procedure for detection of arterial pressure when the applied pressure drops below systolic and diastolic levels. The tail cuff technique is the recognised indirect method for use in the rat (58). Heating of the animal is required in order to vasodilate the tail artery - a thermoregulatory organ in the rat. Only the dilated tail artery can be used for blood pressure measurement.

The advantages of arterial catheterisation are that accurate actual aortic pressures can be measured, both systolic and diastolic pressures are available, and the rapid evaluation of instant changes and continuous monitoring are feasible. The main advantages of the tail

cuff method are that measurements are easily repeatable for long periods, and that the technique is non-invasive. Whilst the main disadvantages of arterial catheterisation apply to long term usage of the technique (and therefore, not to this study), the tail cuff method has a number of inherent drawbacks. These disadvantages include the need to increase body temperature to levels which affect blood pressure in normal animals (further, spontaneously hypertensive rats are more heat susceptible than their normotensive counterparts). Thus the procedure has to overcome the instability of blood pressure during the warming, and the stress of the animal. Tail cuff size also influences blood pressure, as does the position of the cuff in relation to the root of the tail; due to the existance of a pressure gradient along the tail artery.

Based on the above considerations, the direct method appears both more accurate and reliable than the indirect method. Further, arterial catheterisation is central to the technique used to measure cardiac output and renal blood flow throughout this investigation. Arterial catheterisation was therefore adopted to measure blood pressure. It must be stressed however, that in spite of its apparent disadvantages, blood pressure as measured by the tail cuff method shows an excellent correlation with values obtained by arterial catheterisation over a wide range of blood pressure values (Indirect pressure = 0.83 x direct pressure + 14.7. $r = 0.94$, $p < 0.001$ - unpublished data).

2.4.2 Measurement of cardiac output and renal blood flow

Background

In non-primates, the aorta or renal artery may be catheterised, and cardiac output or renal blood flow measured directly by any type of flow meter. Similarly, an electromagnetic or ultrasonic flowmeter may be placed on the aorta or renal artery to measure these variables. However, indirect methods which do not require major surgery are preferable. The majority of these methods utilise the Fick principle. This principle, which states that mass is conserved in the circulation, and that mass is equal to volume multiplied by concentration, enables the prediction that the mass of an indicator injected into the circulation is equal to its concentration in the blood multiplied by blood volume.

A simple example of the evaluation of cardiac output using the Fick principle is illustrated in Fig.2.1. 200 ml of oxygen per minute are absorbed from the lungs into the pulmonary circulation, which can carry 40 ml of this oxygen per l of blood. It follows therefore, that 5 l of blood must have flowed through the lungs per minute to pick up 200 ml of oxygen. This 5 l is a measure of cardiac output, its derivation serving to illustrate one of the several ways by which cardiac output and regional flow may be measured using the Fick principle. Generally speaking, these procedures involve the introduction of an indicator substance, usually either a dye or radiolabelled tracer, into the circulation (in the above example, oxygen was used). A complex array of equipment is required to

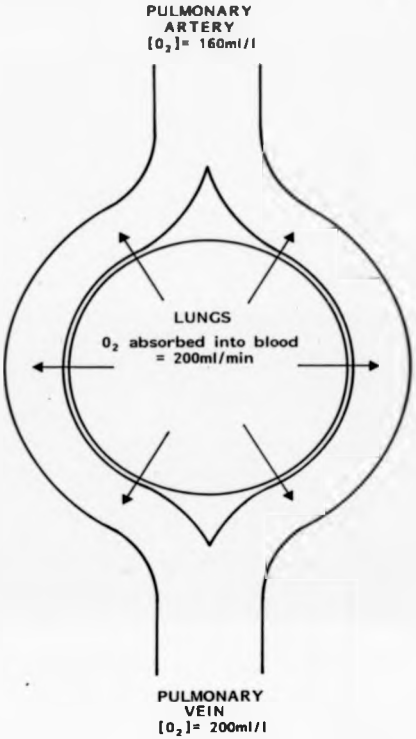


Figure 2.1 Application of the Fick principle to measure cardiac output. (Refer to text)

measure the concentration/activity of the indicator in either blood or tissues. Reliable and accurate estimations of cardiac output can be obtained in this way. When applied to measure regional flow however, theoretical and technical difficulties are often encountered, and the validity of values obtained under such circumstances must be seriously doubted.

The use of radiolabelled microspheres to measure regional flow is a simple and reliable alternative, which overcomes the difficulties previously mentioned, and allows the simultaneous measurement of both cardiac output and regional flow (59). The technique is again an adaptation of the Fick principle. A suspension of radiolabelled microspheres is injected into the left atrium or ventricle of the heart. Here, the microspheres become evenly mixed with the blood and travel with the blood to all organs of the body, where they become impacted in tissue capillary beds. The number of microspheres trapped in an organ will be representative of the blood flow to that particular organ according to the Fick principle:

Thus:

$$q = f. \int_{0}^{\infty} C(t).dt. \qquad \text{——— 1}$$

and:

$$Q = CO. \int_{0}^{\infty} C(t).dt. \qquad \text{——— 2}$$

where:

q = The number of microspheres trapped in the organ

f = Blood flow to the organ - (ml per min)

C = The concentration of microspheres in the blood - (particles per ml)

Q = The number of microspheres injected

CO = Cardiac output - (ml per min)

By dividing 1 by 2 we obtain:

$$\frac{q}{Q} = \frac{f}{CO} \quad \text{or} \quad CO = Q \times \frac{f}{q} \qquad \text{——— 3}$$

As the number of microspheres is proportional to radioactivity, Q and q can be expressed in counts per unit of time. Thus Q/q for a particular organ is found by counting both the radioactivity injected and that present in the organ, using an appropriate counter. To determine cardiac output, the blood flow to and radioactivity of an organ must be known. In practice, a small artery is catheterised, allowing blood to flow into a collecting chamber as required. This chamber acts as a 'reference organ', the blood flow to which can be measured directly. This so-called reference blood sample thus enables the calculation of cardiac output. The blood flow to a particular organ can now be calculated using a modification of equation 3:

$$\text{Organ blood flow (ml per min)} = \frac{\text{Organ counts}}{\text{Reference blood Sample counts}} \times \text{Reference Sample Flow}$$

Before this reference sample microsphere technique can be considered valid for the measurement of cardiac output and regional flow, several important criteria must be met.

Firstly, microspheres must be evenly distributed in the blood and have a similar rheology to red blood cells. Injection of microspheres into the left side of the

heart ensures adequate mixing of blood and microspheres (60) - provided enough microspheres are injected (61). Microspheres have a similar specific gravity to blood (1.30 v. 1.05 g cm^{-3}), and show no evidence of sedimentation in the circulation (62). In addition, there must be no axial streaming; that is, microspheres must not selectively enter arteries of different diameter. Phibbs et al (62), using the rabbit showed that whilst axial streaming of microspheres occurred with large spheres, no streaming was present when smaller 10 μm spheres were used. This study also illustrates the importance of the upper limit of microsphere size in relation to species under study.

Secondly, microspheres with their suspending medium must not in any way alter haemodynamics. Several studies covering a range of species have shown that repeated injections of microspheres do not influence either blood pressure or organ blood flow (63,64), indicating that the injection of microspheres does not effect cardiovascular homeostasis.

Thirdly, all microspheres must become impacted in capillary beds on their first transit of the circulation, and must not recirculate. Sasaki and Wagner (63), measured the extraction efficiency of 50 μm microspheres from the pulmonary and systemic circulations of the rat. When injected intravenously, 100 % of microspheres were trapped in the lungs. When injected into the abdominal aorta, 99.6 % were trapped by systemic capillary beds, with 0.4 % found in the lungs. The lungs therefore, appear to be a reliable indicator of the

degree of recirculation of microspheres, provided the blood supply to the lungs is taken into account. Based on this finding, both 50 and 15 μm microspheres have been shown to show negligible recirculation in the rat (63,64). For studies on the rat, the use of smaller diameter microspheres (15 μm) is advocated, as microspheres of this size are less prone to axial streaming, show a good rheology to red blood cells, occlude less of the vascular bed and are less variable in size. More microspheres can therefore be injected, allowing more reliable measurements of flow to smaller regions.

The use of radioactive microspheres for haemodynamic studies has been the subject of numerous searching investigations, some of which have been mentioned here. Taken together, these studies indicate that microspheres can be used to give reliable and accurate measurements of cardiac output and regional flow in the rat. Further, aside from being both theoretically and practically sound, the use of microspheres in haemodynamic studies confers several advantages over the other techniques previously mentioned.

Firstly, the ideal indicator for measuring the distribution of cardiac output is one with an extraction ratio of 1.0 (65). Microspheres conform to this description as they do not recirculate. Thus provided that the aforementioned criteria are stringently met, the technique is accurate, and errors resulting from the analysis of dye curves and from indicator loss are eliminated. Secondly, the technique is relatively

undemanding technically; a simple peripheral arterial catheter replaces complicated and expensive dye analysis equipment. Thirdly, high quality microspheres of different sizes with different radiolabels are readily available, thus enabling repeated haemodynamic measurements to be made in a single animal (66).

When adapted to the rat however, miniaturisation of the procedure demands that a high level of precision be maintained to avoid the possibility of inducing cardiac instability at the critical moment. If a consistant approach is maintained however, reliable and reproducable haemodynamic data can be expected when using the rat.

Surgical Preparation of Animals

Animals were lightly anaesthetised with Methoxyfluorane (Penthrane, Abbott Laboratories UK) and weighed. The right carotid and left femoral arteries were exposed for catheterisation. The carotid artery was freed from surrounding tissue and nerves for a length of approximately 1.5 cm by careful dissection. A ligature was placed around each end of the isolated section of artery. The distal ligature was tied, and the proximal ligature used to occlude the artery by tension; both procedures preventing subsequent leakage of blood. With the artery under tension a small incision - not greater than a third of the carotid diameter - was made on the anterior face of the vessel, near the distal end of the artery. A polythene catheter (outer diameter - 0.75 mm: Portex, England) filled with heparinised saline (100 units per ml) was carefully inserted 1 cm into the artery and secured in place by means of a third ligature.

The tension on the artery was released by removing the proximal ligature. The femoral artery was catheterised with a polythene catheter (outer diameter - 0.63 mm) in a similar fashion.

Mean arterial pressure (mm Hg) and heart rate (beats per min) were measured by means of the right carotid catheter and displayed on an Elcomatic (Glasgow, Scotland) EM 720 recorder via a Gould (UK) p 323 10 pressure transducer. The carotid catheter was passed retrogradely into the left ventricle for the injection of radiolabelled microspheres: its position being confirmed on the Elcomatic EM 720 recorder. The femoral catheter was used to collect a reference sample of blood by free flow. Body temperature was maintained at 37-38 ^{o}C throughout surgery with the aid of an Animal Thermopad and rectal thermometer (International Market Supply, England).

Determination of cardiac output, renal blood flow and vascular resistance

A 1 ml plastic syringe was used to hold approximately 5 x 10^{4} ^{141}Ce microspheres (15 µm diameter, 10 mCi per g - New England Nuclear, UK) suspended in 0.5 ml of a 0.01 % Tween 80 in 0.9 % saline solution.

The radioactivity within the syringe was counted using a Wilj (England) 2001 gamma counter. The syringe was vigorously shaken for 2 minutes prior to injection using a whirlimixer (Fisons, England), and the microspheres injected manually over 10 seconds via the carotid catheter into the left ventricle. The residual

radioactivity within the syringe was counted and the injected radioactivity calculated. Collection of the reference blood sample commenced immediately prior to injection of microspheres and continued for 60 seconds. The weight and radioactivity of the reference blood sample were measured.

Animals were killed by exsanguination, blood being collected for measurement of the renin-angiotensin system and determination of the packed cell volume, as described elsewhere in this thesis. Immediately after death, both kidneys were carefully dissected from surrounding tissues, removed, weighed and quickly transferred to counting tubes for the measurement of radioactivity.

Calculations

Cardiac output (ml per min) was computed as:

$$\frac{\text{Counts Injected}}{\text{Counts in Reference blood sample}} \times \text{Reference sample blood flow - ml per min (weight of blood collected per min} \div \text{density of blood - 1.107 g per ml)}$$

Renal blood flow (ml per min) was computed as:

$$\frac{\text{Total kidney counts}}{\text{Counts in reference blood sample}} \times \text{Reference sample blood flow - ml per min}$$

Vascular resistance and stroke volume were derived as follows:

$$\text{Total Peripheral Resistance} = \frac{\text{Mean arterial Pressure}}{\text{Cardiac output}}$$

$$\text{Renal vascular resistance} = \frac{\text{Mean arterial pressure}}{\text{Renal blood flow}}$$

The unit of resistance used was dyn cm^{-5} s^{-1} (1mm Hg = 1333.2239 dyn cm^{-2})

Stroke volume = Cardiac Output

Heart rate

All values were related to either body or kidney weight as appropriate.

2.4.3 Measurements of the Renin Angiotensin System

Background

Advances in understanding the renin-angiotensin system have quite often resulted from an advance in measurement. The first attempt at standardisation of assay took place in Goldblatt's laboratory, with the definition of the renin unit. The Goldblatt unit was defined as the amount of enzyme necessary to raise the blood pressure of a dog of certain weight by 30 mm Hg. Biological assays of this type, in which the end point is either a rise in blood pressure in the intact animal or the contraction of smooth muscle in an isolated organ, formed the basis of renin and angiotensin measurements until the development of the technique of radioimmunoassay. This revolutionised assay methods in that it allowed for widespread standardisation, offered an increased degree of precision, and allowed measurements to be made from small volumes of plasma (important when studying small mammals such as the rat).

Measurements of the renin-angiotensin system, using a radioimmunoassay comprise two main stages. Firstly, the specimen to be analysed (either plasma or renin-containing tissue) is incubated, at both temperature and pH optimums for renin, in the presence of inhibitors to both

angiotensin converting enzyme and angiotensinases. These inhibitors prevent the loss of angiotensin I to measurement, through its conversion to the non-cross-reactive angiotensin II, and through enzymatic degradation, respectively. Secondly, the angiotensin I generated from the above incubation is quantitated by radioimmunoassay.

Modifications in the incubate from the first stage allows the simultaneous measurement of the renin-angiotensin variables of interest to this investigation - through the radioimmunoassay of angiotensin I.

These variables are namely:

Plasma Renin Substrate Concentration - Amount of angiotensin generated after the addition of excess exogenous renin.

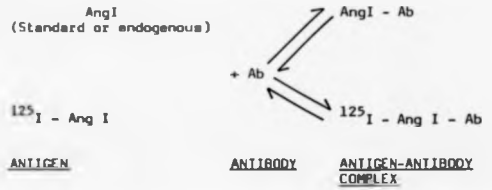
Plasma Renin Activity - Amount of angiotensin generated in plasma alone, thereby reflecting the endogenous concentrations of renin and its substrate.

Plasma and Renal Renin Concentration - Reflects the enzymatic activity of renin after the addition of an excess of exogenous renin substrate, thus allowing the measurement of renin activity independantly of variations in renin substrate.

It is not the province of this section to review the general method of radioimmunoassay. A detailed discussion of the principle may be found in Homey and Haber (67). In brief, the method depends on perturbing the equilibrium

between a labelled antigen and a specific antibody by the unknown antigen to be measured. The antibody used should have an equal affinity for the labelled and unknown antigen. The antibody must be of sufficiently high affinity for measurement of picomolar concentrations of the peptide, and of sufficient specificity so that there is no cross reaction with other plasma components, particularly angiotensinogen (renin substrate). The labelled antigen must be highly purified, and must have undergone radiolabelling without any impairment of its antibody binding properties.

In practice, the unknown antigen is the angiotensin I generated from the first stage of the procedure, whose radioimmunoassay can be described by the following equation:



The unknown and I-labelled angiotensin I compete for a limited number of antibody binding sites. As the concentration of one antigen increases, the fraction of the other antigen bound to the antibody decreases. The amount of labelled angiotensin I bound to the antibody is therefore inversely related to the concentration of angiotensin I in the unknown or standard, and serves as an index of angiotensin I concentration. This antigen-antibody complex can be separated from unbound labelled antigen using a number of procedures. The radioactivity of either free or bound antigen is counted, from which the concentration of angiotensin I in the unknown or standard may be calculated. In this fashion, using known amounts of angiotensin I, a standard curve is contructed, from which the concentration of angiotensin I in unknown specimens is found.

Materials

All laboratory chemicals were obtained from either BDH (England) or Sigma (UK), unless otherwise stated.

Buffers

Made up in distilled deionised water and stored at 4° C.

Incubation Buffer

0.12 mol per l disodium hydrogen orthophosphate with 0.015 mol per l ethylenediaminetetra-acetic acid (EDTA: disodium salt), adjusted to pH 6.5 with sodium dihydrogen orthophosphate.

Tissue Homogenisation Buffer

0.1 mol per l sodium chloride, 0.05 mol per l disodium hydrogen orthophosphate, 5 mmol per l Na_2EDTA, 5 mmol per l 8-hydroxyquinoline and 2 mmol per l

phenylmethylsulphonyl fluoride (from a stock solution of 50 mmol per l ethanol); adjusted to pH 7.5 with sodium dihydrogen orthophosphate.

The Na_2 EDTA was dissolved in the minimum amount of water necessary and the 8-hydroxyquinoline added. Add PMSF and previously prepared phosphate buffer dropwise, with continual stirring, and heat if necessary.

Radioimmunoassay Buffer

0.1 mol trizma base with 3 g bovine serum albumin and 2 g neomycin sulphate (Biorex Laboratories Ltd, London) per litre, adjusted to pH 7.5 with glacial acetic acid.

Angiotensin Converting Enzyme Inhibitor and Anticoagulant

EDTA - dipotassium salt.

Anti-angiotensinases

8-hydroxyquinoline (8-OHQ), phenylmethylsulphonyl fluoride (PMSF)and 2,3 - dimercaptopropanol (British Anti-Lewisite: BAL). Inhibitors were dissolved in ethanol in the required combinations, to give a final concentration of inhibitor in plasma.

Angiotensin I Standards

A 9 µg vial of medical research standard A, 71/328 for angiotensin I (asp^1 leu^5) synthetic (National Institute for Biological Standards and Control, London) was used to produce eight standards of 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 pg angiotensin I per 200 µl radioimmunoassay buffer, by serial dilution. Standards were dispensed into plastic assay tubes in 200 µl aliquots, capped and stored at -20°C.

Labelled Angiotensin I

10 µCi (3-[^{125}I] iodotyrosyl^{4}) angiotensin I (2000 Ci per mmol - Amersham, UK) was diluted in radioimmunoassay buffer to give approximately 75 x 10^{4} counts per minute (cpm) per ml, at the quoted reference date. 2 ml aliquots of this diluent were stored at -20°C. On the day of assay, the required number of aliquots were diluted 1:5 in radioimmunoassay buffer to give 15 x 10^{3} cpm per 100 µl.

Angiotensin I Antibody

1 ml vials of rabbit angiotensin I antiserum (New England Nuclear, UK) were stored at 4°C. On the day of assay the required number of vials were each diluted to 45 ml with radioimmunoassay buffer.

Dextran-Coated Charcoal Suspension

3 g charcoal (Hopkin and Williams, England) and 2.5 g dextran T-70 (Pharmacia Fine Chemicals, UK) per litre distilled deionised water.

Renin Substrate

Nephrectomised rat plasma is a rich source of renin substrate, and has a negligible renin activity (68). Large adult male Sprague-Dawley rats (~350g) were bilaterally nephrectomised under Methoxyfluorane anaesthesia. 18-24 hours post-nephrectomy animals were reanaesthetised, and 6-8 ml blood withdrawn from each rat by cardiac puncture. Blood was taken into plastic syringes - containing 0.05 volume of a 100 g per l solution of K_2EDTA, and pooled in a plastic receptacle placed on ice.

Blood was centrifuged at 3000 rpm for 10 minutes at 4°C.

Plasma was decanted off and placed in visking dialysis tubing (Scientific Industries International Inc., UK). Sacs were dialysed against ~ 50 volumes of incubation buffer pH 6.5, for 17 hours at 4°C. Renin substrate rich plasma was stored in 100 µl aliquots at -20°C.

Renin

Purified renin extract was obtained from bovine renal tissue. Two fresh cow kidneys were obtained from a local slaughterhouse, chilled, and stored at -20°C. Renin was extracted and purified from thawed outer cortex lobes after the method of Haas, et al (procedure A) (69). Purified renin extract was stored in 100 µl aliquots at -20° C.

Methods

Unless otherwise stated, all procedures were carried out at 4 °C, using plastic, standard 3 ml assay tubes (Luckhams, UK), which were shaken with a Whirlimixer (Fisons, England) after each addition.

Stage I - Generation of Angiotensin I.

All incubations were performed in a waterbath (Gallenkamp, UK) at 37°C. Reactions were stopped by plunging incubates into ice-cold water.

Plasma Renin

Immediately after collection, blood used to measure plasma renin activity, concentration and substrate concentration (approximately 3 ml) was added to EDTA (10:1 volume of a 100 g per l solution of the dipotassium salt), chilled and centrifuged at 3000 rpm for 10 minutes.

Plasma was decanted off and stored at -20°C until required.

On the day of assay, samples were allowed to thaw slowly on ice. For each plasma, renin activity, concentration and substrate concentration were measured from the same assay - to eliminate variations which may result from repeated freezing and thawing of plasma (70).

Plasma Renin Activity

250 µl plasma was added to an equal volume of incubation buffer. 5 µl anti-angiotensinase cocktail was added, containing PMSF and BAL, to give a final concentration of inhibitor in plasma of 1 and 4.7 mmol per l respectively. The 505 µl incubate was split into three equal portions, which were incubated in capped assay tubes for 0, 20 and 30 minutes respectively. 10 µl of each incubate was used for radioimmunoassay of angiotensin I as described in Stage 2.

Plasma Renin Concentration

5 µl PMSF/BAL cocktail (final concentrations in plasma: 1 and 4.7 m mol per l respectively) was added to 100 µl binephrectomised rat plasma. Add 25 µl plasma and buffer with 125 µl incubation buffer. The 255 µl incubate was split and incubated as for plasma renin activity. Similarly, 10 µl of each incubate was used for stage 2.

Plasma Renin Substrate Concentration

300 µl incubation buffer, containing 8-OHQ (final concentration in plasma: 17 mmol per l), was added to 100 µl bovine renin extract. 160 µl of this mixture was added to 40 µl plasma. 80 µl portions of the incubate

were incubated for 0 and 60 minutes.

80 µl of a 10 % solution of trichloroacetic acid was added to each incubate, which were then centrifuged at 3000 rpm for 5 minutes. 80 µl supernatant was added to 1,420 µl of a mixture of radioimmunoassay buffer (50 ml) and 1 M sodium hydroxide (1.116 ml). 200 µl of this mixture was assayed in stage 2.

Renal Renin Concentration

Renal cortex (approximately 50 mg) was frozen immediately after removal, and stored at -20°C prior to measurement of renal renin concentration. Cortex samples were homogenised in homogenisation buffer (10 mg cortex per ml buffer), using 10 ml glass homogenisation tubes and teflon pestles (Scientific Industries International Inc. (UK)) and a Tri-R K41 homogeniser. The homogeniser was set at half speed, and 3 x 10 second passes used to effect homogenisation. Homogenates were transferred to 20 ml plastic tubes (Sterilin, England) and centrifuged at 3000 rpm for 10 minutes to remove tissue debris. Supernatants were decanted off, and 200 µl samples of each supernatant stored at -20°C, for measurement of protein content at a future date.

5 µl PMSF/8-OHQ cocktail (final concentrations in supernatant: 1 and 5 mmol per l respectively) was added to 100 µl binephrectomised rat plasma. 10 µl supernatant was added to the mixture. The 115 µl incubate was split into three portions which were incubated for 0, 15 and 30 minutes respectively.

15 and 30 minute incubates were diluted either x 20 or x 50, as required with radioimmunoassay buffer. 10 µl

of each incubate was used for stage 2.

Stage 2 - Radioimmunoassay of angiotensin I

Assays were performed using duplicates of each determination.

Assay tubes 1-24 enable a standard curve to be constructed.

Tubes 1-3 contain 400 µl radioimmunoassay buffer and 100 µl labelled angiotensin I - TOTALS.

Tubes 4-5 contain 200 µl radioimmunoassay buffer and 200 µl angiotensin I antibody. Here, there is no antibody to bind specifically to the antigen (angiotensin I). Instead, antibody will bind non-specifically to the walls of the tube, separation agent, etc - BLANK.

Tubes 6-8 contain 200 µl radioimmunoassay buffer, 200 µl angiotensin I antibody and 100 µl labelled angiotensin I. Here, there is no competition between labelled and unlabelled antigen. The concentration of unlabelled antigen is zero and the maximum amount of labelled antigen will be bound - Bo.

Tubes 9-24 contain the eight unlabelled angiotensin I standards, in increasing concentrations, 200 µl angiotensin I antibody and 100 µl labelled angiotensin I - STANDARDS.

Tubes 25 onwards contain the appropriate volume of unknown plasma or renal cortex supernatant, (Volumes were calculated so as to ensure that the amount of angiotensin I generated from Stage I was within the working range of the standard curve), 200 µl radioimmunoassay buffer, 200 µl angiotensin I antibody and 100 µl labelled angiotensin I - UNKNOWNS.

Assay tubes were allowed to incubate for approximately 15 hours prior to separation of bound and unbound fractions.

Separation Procedure

1 ml of stirred dextran-coated charcoal suspension was added to each assay tube. Within 5 minutes, tubes were centrifuged at 3000 rpm for 5 minutes. The bound (supernatant) and free (charcoal pellet) fractions were separated by aspiration, using a vacuum pump, and the radioactivity of the charcoal pellet counted in either a Wilj 2001 or LKB (England) Clini Gamma gamma counter.

Data Reduction

Data obtained from the counters were analysed using a Hewlett Packard (UK) - 97 programmable printing calculator and radioimmunoassay programmes.

The radioactivity of TOTALS, BLANK and Bo was used to express the radioactivity of STANDARDS as the percentage of bound antigen (B) to Bo, that is, B/Bo. For each standard, B/Bo was plotted against the logarithm of the concentration of the standard. The resultant sigmoidal linear-logarithmic curve was transformed into a straight line by interpolation, using a logit transformation. The formula for transforming B/Bo to the corresponding logit is:-

$$\text{logit } (B/Bo) = \text{Ln } \frac{B}{Bo - B}$$

A logit plot in effect, stretches out the flatter parts of the sigmoidal B/Bo logarithm concentration plot, to create an approximately linear plot - Fig.2.2.

A least squares regression line on a logit B/Bo - logarithm concentration plot was used to fit a straight

$B/_{Bo}$ = Fraction of bound labelled antigen. Logit $B/_{Bo} = Ln \frac{B}{Bo - B}$

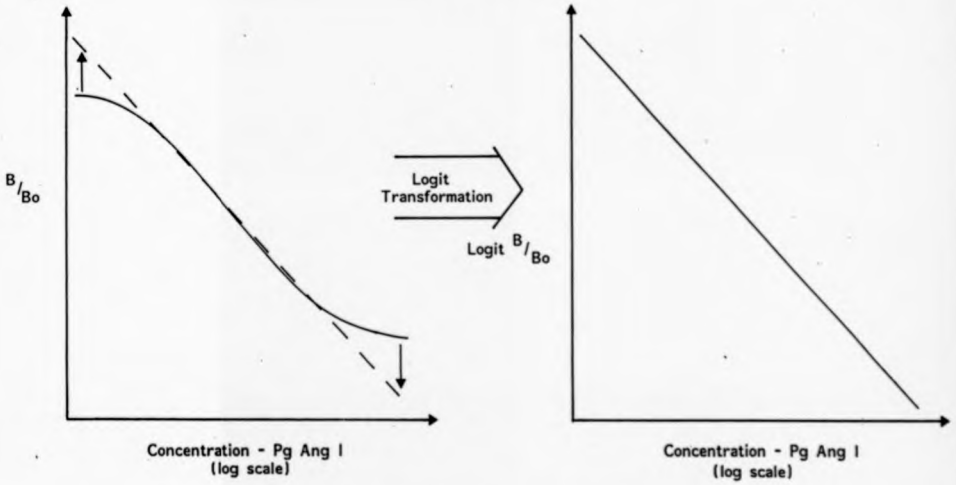


Figure 2.2 illustrates the effect of the logit transformation on a sigmoidal $B/_{Bo}$ - log concentration plot (refer to text)

line to the standard curve (71).

The radioactivity of UNKNOWNS was used to calculate a concentration of angiotensin I in pg from the standard curve.

Renin activity and concentration were expressed as ng angiotensin I ml^{-1} plasma $hour^{-1}$, and renin substrate concentration as ng angiotensin I per ml plasma. Renal renin concentration was initially expressed as ng angiotensin I per hour. Values obtained from UNKNOWNS must therefore be converted from pg angiotensin I to the appropriate form. The following derivation, using plasma renin activity (PRA) as an example, illustrates how this was achieved:

i) pg Ang I ⟶ ng Ang I $\times 10^{3}$ —— 1

ii) Ang I per x µl plasma ⟶ Ang I per ml plasma

x = volume of plasma contained within the 10 µl incubate used for assay

= 4.95 µl

Therefore:

Ang I per 4.95 µl ⟶ Ang I per ml $\times \frac{1000}{4.95}$ —— 2

iii) Ang I per 20 min - T20 ↘

Ang I per hr $\frac{3(T20) + 2(T30)}{2}$ — 3

Ang I per 30 min - T30 ↗

Combining 1,2 and 3 we have:

$$PRA = \frac{1}{4.95} \times \frac{3(T20) + 2(T30)}{2} \text{ ng Ang I } ml^{-1} \text{ } hr^{-1}$$

Quality Control

Immunological assays are subject to errors arising from the instability of reagents and the involvement of complex manipulations such as dilution and separation steps (72). Thus it is necessary to adopt control procedures in order that assay performance be maximised and then maintained within acceptable limits. This is achieved by measuring the precision and reproducibility of assays.

Errors in radioimmunoassay arise from firstly, sample preparation and secondly, the random nature of radioactive decay. Preparation error includes errors due to pipetting, mixing, incomplete separation of bound and free fractions, dilutions etc. Further, a large preparation error in duplicates may effect the fitting of the standard curve and therefore, the evaluation of the whole assay.

The precision of assays was evaluated by the inclusion of control samples in the assay procedure, which permitted the measurement of both intra- and inter-assay variation. Intra-assay variation was measured by the addition (to stage 1) and subsequent recovery (from stage 2) of known amounts of angiotensin I. By adopting this procedure, intra-assay variation was 104 $\pm$ 8 (mean $\pm$ SEM ; n = 7), 100 $\pm$ 5 (n = 6) and 126 $\pm$ 3 % (n= 3), for plasma renin activity, concentration and substrate concentration assays, respectively. Inter-assay variation was measured from control samples of either pooled plasma or pooled renal cortex supernatant, which were incubated and assayed as

for UNKNOWNS. From this procedure, inter-assay coefficients of variation were found to be 6.9 % at 52 pg angiotensin I (n = 20) and 12.7 % at 36 pg angiotensin I (n = 9), for plasma and renal renin assays respectively.

The concentrations of angiotensin I used to assess intra- and inter-assay variability were in the working range (linear region) of the B/Bo - logarithm concentration plot. Further, the generation of angiotensin I from stage I incubations showed a linear increase with time. This important observation shows the efficiency with which anti-angiotensinases prevent the loss of angiotensin I to measurement.

The reproducibility of assays was monitored from information inherent to the assay procedure, such as data from replicates and the standard curve. Values showing an obvious deviation from the appropriate replicate (a good estimation of preparation error) were discarded. The profile of each B/Bo - logarithm concentration plot was analysed together with data from each regression line on the logit B/Bo - logarithm concentration plot. Assays were rejected if either the B/Bo - logarithm concentration plot profile deviated from normal, or if the regression line on the logit of the above plot yielded a correlation coefficient of < 0.999.

2.4.4 **Measurement of Protein Concentration**

Background

The measurement of renal renin concentration - from homogenised renal cortex samples - has been described in the preceding section. Renin concentration was expressed as ng angiotensin I generated per hour. Tissue enzyme

concentrations are however, conventionally related to either the weight or protein content of tissue. Due to the possibility that renal cortex samples may have become dehydrated whilst stored at -20 °C (a view supported by the observation of the presence of ice crystals surrounding certain specimens), it was considered inappropriate to relate renal renin to cortex weight. A measurement of protein content on the other hand, is widely recognised as a more consistant, and therefore reliable standard, to which tissue enzymes such as renin, may be related. Thus the protein concentration of renal cortex homogenates was measured, in order to relate renal renin concentration to the protein content of the corresponding renal cortex sample.

A preliminary experiment designed to measure the protein content of a pooled sample of renal cortex homogenate, using the Lowry method (73), clearly indicated that the buffer in which cortex samples had been homogenised was interfering with the procedure. This interference probably resulted from the presence of EDTA and 8-hydroxyquinoline in the buffer.

Precipitation of the protein contained in renal cortex homogenates by the combined use of sodium deoxycholate and trichloroacetic acid, is a convenient one-step method of separating protein from interfering reagents (74). The precipitated protein can then be measured using the standard Lowri method.

Materials

All reagents (made up in distilled deionised water, unless otherwise stated) were obtained from either BDH

(England) or Sigma (UK).

Phosphate Buffer, pH 7.5 (see incubation buffer: p32)

Protein Standards

25 mg bovine serum albumin was dissolved in 25 ml phosphate buffer. This stock solution was used to prepare eight standards of 1000, 750, 500, 375, 250, 125, 100 and 0 µg of protein per ml, by dilution in the phosphate buffer.

Precipitation Agents

2 % sodium deoxycholate

20 % trichloroacetic acid

Reagent A

1 ml 20 g per l sodium potassium tartrate

1 ml 10 g per l copper sulphate

Made up to 100 ml with 20 g per l sodium carbonate in 0.1N sodium hydroxide.

Reagent B

1N Folin-Ciocalteu phenol reagent

Methods

Unless otherwise stated, all procedures were carried out at room temperature in plastic, 4 ml assay tubes (Luckhams, UK), which were shaken with a Whirlimixer (Fisons, England) after each addition.

On the day of assay, renal cortex homogenates (see p37) were thawed and diluted 1:5 in phosphate buffer. This dilution factor was found to give protein values in the working range of the standard curve, when the following assay procedure was applied to each sample:

50 µl sample (either standard or diluted renal cortex homogenate) was adjusted to 3 ml with distilled deionised

water. Add 25 µl sodium deoxycholate and allow to stand for 15 minutes. 1 ml trichloroacetic acid was added, and the mixture centrifuged at 3,300 rpm for 30 minutes. The supernatant was removed by aspiration, and 1 ml reagent A added to the protein pellet. After 10 minutes, 100 µl reagent B was added, and the mixture allowed to stand for at least 45 minutes.

After 1 hour, samples were transferred to cuvettes (light path:1 cm, Sarstedt, UK), and absorbance measured at a wavelength of 750 nm, using a CE = CIL (England) 5093 double beam spectrophotometer.

Data Reduction

The theory behind Folin phenol quantitation of protein is well documented (75) and need not be detailed here. Briefly, the non-linear relationship between protein concentration and absorbance can be accurately described by a power function of the form:

$P = (A/a)^{1/b}$

where: P = protein concentration, A = absorbance, and a and b are constants.

In practice, a Hewlett Packard (UK) - 97 programmable printing calculator and curve fitting programme were used to construct the linear absorbance-concentration plot from which the protein concentration of renal cortex homogenates (µg per ml) was calculated. These values were used to equate renal renin concentration (ng angiotensin I per hr) to protein content (per mg) as follows:

Using the above procedure 1000µl cortex homogenate was found to contain x µg protein

During stage 1 of the radioimmunoassay procedure used to measure renal renin concentration, 10 µl of the above homogenate was added to 105 µl of renin substrate and inhibitors. 10 µl of this mixture was assayed for angiotensin I, and contained $\frac{x}{100} \times \frac{10}{115}$ µg of protein (y µg protein)

This 10 µl was found to have generated x ng angI per hr, and therefore contained:

x x $\frac{1000}{y}$ ng ang I per mg of renal cortical protein per hr.

2.4.5 **Measurement of Packed Cell Volume**

The control of extracellular fluid volume is critical to the maintenance of cardiovascular homeostasis. For example, acute and severe haemorrhage will induce a fall in both arterial pressure and renal blood flow, resulting in shock. The renin-angiotensin system plays a central role in the control of extracellular fluid volume, principally through the stimulatory effect of angiotensin II on the adrenal cortex (aldosterone) (76).

A change in the packed cell volume (haematocrit) of blood is widely recognised as a reliable reflection of a change in plasma volume (77). A sample of blood was therefore taken from each animal as described previously. Blood was taken into potassium EDTA, and the packed cell volume measured using a coulter S+ analyser.

2.5 **Statistics**

Data obtained from experimental groups of animals were initially tested for normality of distribution. For each variable, those data found to be normally distributed were

presented as a mean ± 95 % confidance limits (eg. 125 ± 12) (78). A median and range (eg. 6.7; 1-17) were used to describe those data found to be non-normally distributed.

The significance of differences between groups of animals was tested using the expedient parametric (one way analysis of variance (ANOVA) or paired t-test) or non-parametric (Kruskal-Wallis or Wilcoxon rank sum test) test. Where appropriate, the relationship between two variables has been investigated by means of either a correlation or regression analysis (79). The probability value of the respective test statistics F, t, H, Z and r has been used to express the level of significance of differences or relationships as follows: NS, non significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Chapter 3

INFLUENCE OF OESTROGEN ON RENAL AND SYSTEMIC HAEMODYNAMICS THE RENIN-ANGIOTENSIN SYSTEM AND PLASMA VOLUME STATUS IN THE NORMOTENSIVE RAT

3.1 INTRODUCTION

The kidney is one of the main organs concerned with the maintenance of cardiovascular homeostasis. This is achieved by several interrelated systems including those involved in extracellular fluid volume regulation as well as the production and/or activation of vasoactive compounds such as angiotensin, prostaglandins and kinins. Haemodynamic disturbances, such as hypertension, are common to many types of renal disorders. However, the mechanisms whereby such disturbances are maintained by structural and functional intrarenal changes are poorly understood. Likewise, the influence of alterations in systemic haemodynamics upon intrarenal autoregulatory mechanisms, especially those governing blood flow, remain obscure.

Oestrogen can perturb normal cardiovascular function, as evidenced by the occurrence of hypertension in women ingesting oestrogen-containing oral contraceptives (33,34). Further, it is clear that oestrogen may have the capacity to influence the control of the renal circulation (see p 11). To date however, there have been no detailed studies of the role of the renal circulation and its control mechanisms in relation to changes in systemic haemodynamics during oestrogen treatment.

The objective of this study therefore, was to assess the impact of oestrogen on both systemic and renal haemodynamics, the renin-angiotensin system and plasma volume status in the rat. By studying the archetypal intrarenal hormonal vasopressor system, it may be possible to obtain a better understanding of the role of the renal circulation in relation to changes in systemic haemodynamics during the pharmacological challenge of oestrogen treatment.

3.2 METHODS

Adult female Sprague-Dawley rats (Nottingham University) weighing 190-240g were used throughout the study. Animals were housed at 21 ± 1°C, fed with Special Diet Services (England) rat and mouse number 3 pellets (Sodium content: 0.34%: Potassium content: 0.74%) and allowed free access to tap water.

Oestrone acetate in ethyl oleate (4 mg per ml) was administered by subcutaneous injection to two groups of animals.

Group A received a total of 5 mg oestrogen: given as either 0.5 mg per day for 10 days, or 1 mg on alternate days for 10 days. Group B received a total of 10 mg oestrogen: given as either 1 mg per day for 10 days, or 1 mg on alternate days for 20 days. Control animals were given an appropriate injection of vehicle alone. All animals were weighed daily whilst receiving injections.

24-hours after receiving their last injection of either oestrogen or vehicle, animals were prepared for experimental analysis. This surgical preparation, together with the procedures which were subsequently applied to measure systemic and renal haemodynamics, plasma and renal renin, and packed cell volume, have been fully described in Chapter 2.

3.3 RESULTS

The injection of vehicle alone did not alter any of the parameters under study (Table 3.1). For the purpose of clarity therefore, data obtained from vehicle injected animals has been pooled, and referred to as the control group against which the oestrogen treated groups have been compared. Likewise, data from oestrogen treated animals has been presented in relation to the total dose of oestrogen administered, since the frequency of dosage was found to be irrelevant. Group A therefore relates to

animals who received a total of 5 mg oestrogen, and group B to animals who received a total of 10 mg oestrogen.

Haemodynamics (Figure 3.1 and Table 3.2)

Renal Blood Flow (RBF)

Renal Blood Flow fell from a control value of 5.65 ± 0.33 ml $g^{-1}min^{-1}$ to 4.71 ± 0.41 ml $g^{-1}min^{-1}$ in group B ($p< 0.01$), but remained unchanged to group A at 5.11 ± 0.59 ml $g^{-1}min^{-1}$.

Mean Arterial Pressure (MAP)

Oestrogen treatment resulted in a rise in MAP from a control value of 120 ± 2 mm Hg to 129 ± 4 ($p< 0.001$) and 136 ± 4 ($p < 0.001$) mm Hg in groups A and B respectively. The rise in MAP induced by oestrogen in group B was significantly larger than that noted in group A ($p< 0.05$).

Cardiac Output (CO)

Values for CO in group A and B were 239 ± 24 and 278 ± 37 ml $min^{-1} kg^{-1}$ respectively. These values did not differ significantly from that recorded in the control group of animals (252 ± 16 ml $min^{-1} kg^{-1}$).

Total Peripheral Resistance (TPR)

TPR remained unchanged as a result of oestrogen treatment, with values of 39,482 ± 2,705, 44,271 ± 4,622 and 42,258 ± 443 dyn $cm^{-5} sec^{-1} kg^{-1}$ in controls, group A and group B respectively.

Renal Vascular Resistance (RVR)

RVR rose from a control value of 1,764 ± 133 dyn $cm^{-5} sec^{-1} kg^{-1}$ kidney to 2,067 ± 127 ($p< 0.05$) and 2,219 ± 267 ($p< 0.001$) dyn $cm^{-5} sec^{-1} kg^{-1}$ in groups A and B respectively.

Plasma and Renal Renin (Figure 3.1 and Table 3.2)

Plasma Renin Activity (PRA)

PRA increased from a control value of 8.35 ± 1.22 ng Ang I $ml^{-1}hr^{-1}$ to 12.67 ± 1.59 ng Ang I $ml^{-1}hr^{-1}$ in group B ($p<0.001$)

but remained unchanged in group A at 6.97 ± 1.39 ng Ang I $ml^{-1} hr^{-1}$. The value for PRA observed in group B was significantly greater than that observed in group A ($p < 0.001$).

Plasma Renin Concentration (PRC)

Oestrogen reduced PRC from a control value of 102.4 ± 14.1 ng Ang I $ml^{-1} hr^{-1}$ to 54.3 ± 14.3 ($p < 0.001$) and 33.7 ± 4.7 ($p < 0.001$) ng Ang I $ml^{-1} hr^{-1}$ in groups A and B respectively. The decrease in PRC noted in group B was significantly greater than that noted in group A ($p < 0.01$).

Plasma Renin Substrate Concentration (PRS)

PRS increased in the oestrogen treated rat from a control value of 230 ± 16 ng Ang I ml^{-1} to 424 ± 69 ($p < 0.01$) and 610 ± 86 ($p < 0.001$) ng Ang I ml^{-1} in groups A and B respectively. The rise in PRS noted in group B was significantly greater than that noted in group A ($p < 0.05$).

Renal Renin Concentration (RRC)

Oestrogen reduced RRC from a control value of 10,222 ± 1,060 ng Ang I mg^{-1} renal cortical protein hr^{-1} to 6,473 ± 1,368 ($p < 0.001$) and 5,544 ± 770 ($p < 0.001$) ng Ang I mg^{-1} renal cortical protein hr^{-1} in groups A and B respectively. There was no difference in RRC between groups A and B.

Packed Cell Volume (PCV)

A fall in the PCV occurred as a result of oestrogen treatment, from a control value of 36.7 ± 0.6 % to 33.0 ± 1.4 ($p < 0.001$) and 32.5 ± 0.6 % ($p < 0.001$) in groups A and B respectively.

Body Weight

Control animals gained an average of 0.8 g per day ($n = 18$), whereas animals in groups A and B lost 1.35 ($n = 17$) and 2.1 g per day ($n = 14$) respectively. This loss in body weight induced by oestrogen is in agreement with published data (80).

Relationships Between Oestrogen Induced Changes in Haemodynamics, PRA and PCV

The fall in RBF induced by oestrogen in group B took place with minimal changes in both CO and TPR, but was accompanied by significant rises in RVR, MAP and PRA.

From Figure 3.2 it can be seen that in the oestrogen treated animal, the percentage increase in RVR exceeds that in TPR; this difference being greatest in group B where the ratio of change in RVR to TPR was 5.3:1.

Although an inverse correlation between RBF and PRA was noted in both controls (RBF = - 0.144.PRA + 6.77; r = - 0.454; n = 20; $p < 0.02$) and group A (RBF = - 0.208.PRA + 6.61; r = - 0.488; n = 17; $p < 0.05$), no such relationship was found in group B (Fig.3.3).

An inverse correlation was observed between PCV and MAP (PCV = - 0.107.MAP + 48.7; r = - 0.354; n = 55; $p < 0.01$) when the data from all experimental animals were considered together (Fig.3.4). No relationship was noted between RBF and MAP in any experimental group. Likewise, there was no relationship between MAP and PRA in any experimental group.

3.4 DISCUSSION

The findings from this study have demonstrated that whereas oestrone acetate, given as a total dose of 10mg, resulted in a significant fall in renal blood flow in the normotensive rat, no change in renal blood flow occurred with a total dose of 5 mg. This effect of oestrogen could not be accounted for by a change in cardiac output, but was accompanied by a 37% increase in renal vascular resistance. Both oestrogen regimes resulted in a significant rise in mean arterial pressure, which was dose dependant, and associated with a significant fall in the packed

TABLE 3.1 Experimental Data for Non Treated and Vehicle (ethyl oleate) Injected Control Rats. Values are either means + 95% C.L. or a median and range. Results of statistical analysis of the data are shown in the test column. In this column, an F value indicates the use of a one way ANOVA and an H value the Kruskall-Wallis test. NS = non significant: n = number of animals.

	Control for Group A		Control for Group B		Non Treated		Test
Mean Arterial Pressure (mmHg)	120 ± 3,	n = 8	124 ± 5,	n = 18	117 ± 4,	n = 9	F = 1.92, NS
Heart Rate (beats min^{-1})	394 ± 24,	n = 8	400 ± 10,	n = 18	397 ± 20,	n = 5	F = 0.14, NS
Cardiac Output (ml $min^{-1}kg^{-1}$)	283 ± 33,	n = 8	251 ± 22,	n = 18	240 ± 25,	n = 9	F = 2.06, NS
Stroke Volume (ml $beat^{-1}kg^{-1}$)	0.72 ± 0.10,	n = 8	0.63 ± 0.05,	n = 18	0.57 ± 0.04,	n = 5	F = 3.22, NS
Renal Blood Flow (ml $min^{-1}g^{-1}$)	5.77 ± 0.73,	n = 8	5.94 ± 0.53,	n = 18	5.35 ± 0.82,	n = 9	F = 0.78, NS
Renal Vascular Resistance (Dyne $cm^{-5}sec^{-1}kg^{-1}$)	1,709 ± 220,	n = 8	1,715 ± 137,	n = 18	1,718 (1,315 - 3,154)	n = 9	H = 0.93, NS
Total Peripheral Resistance (Dyne $cm^{-5}sec^{-1}kg^{-1}$)	34,772 ± 4,500,	n = 8	40,974 ± 4,287,	n = 18	39,890 ± 4,416,	n = 9	F = 1.64, NS
Plasma Renin Activity (ng Ang I $ml^{-1}hr^{-1}$)	6.51 ± 1.20,	n = 8	9.21 ± 1.51,	n = 17	7.88 ± 0.68,	n = 5	F = 2.86, NS
Plasma Renin Concentration (ng Ang I $ml^{-1}hr^{-1}$)	89.8 ± 19.0,	n = 8	109.0 ± 17.1,	n = 12	97.9 ± 26,	n = 5	F = 2.05, NS
Renal Renin Concentration (ng Ang I $mg^{-1}hr^{-1}$)	9,012 ± 1,605,	n = 7	11,225 ± 1,107,	n = 18	11,192 ± 2,435,	n = 4	F = 3.75, NS
Packed Cell Volume (%)	36.1 ± 0.6,	n = 8	37.4 ± 0.6,	n = 18	37.9 ± 1.6,	n = 5	F = 1.43, NS

TABLE 3.2 Data relating to Systemic and Renal Haemodynamics and the Renin Angiotensin System in the Oestrone Acetate Treated and Control Groups of Rats. Group A received 5 mg of the oestrogen and group B 10 mg. Values are means ± 95% confidence limits, n = number of animals in each group. Data was analysed using a one way analysis of variance. * $p<0.05$; ** $p<0.01$; *** $p<0.001$: Significantly different from controls.

Parameter	Controls		Group A		Group B	
Mean Arterial Pressure (mmHg)	120 ± 2	n = 35	129 ± 4 ***	n = 17	136 ± 4 ***	n = 19
Heart Rate (beats min^{-1})	393 ± 8	n = 31	365 ± 14 ***	n = 17	371 ± 12 **	n = 19
Cardiac Output (ml $min^{-1}kg^{-1}$)	252 ± 16	n = 35	239 ± 24	n = 17	278 ± 37 *	n = 19
Stroke Volume (ml $beat^{-1}kg^{-1}$)	0.64 ± 0.04	n = 31	0.66 ± 0.05	n = 16	0.75 ± 0.10 **	n = 19
Renal Blood Flow (ml $min^{-1}g^{-1}$)	5.65 ± 0.33	n = 35	5.11 ± 0.59 *	n = 17	4.71 ± 0.41 ***	n = 19
Renal Vascular Resistance (Dyne $cm^{-5}sec^{-1}kg^{-1}$)	1,764 ± 133	n = 35	2,067 ± 127	n = 16	2,419 ± 267	n = 19
Total Peripheral Resistance (Dyne $cm^{-5}sec^{-1}kg^{-1}$)	39,482 ± 2,705	n = 35	44,271 ± 4,622	n = 16	42,258 ± 443 ***	n = 13
Plasma Renin Activity (ng Ang I $ml^{-1}hr^{-1}$)	8.35 ± 1.22	n = 25	6.97 ± 1.39 ***	n = 18	12.67 ± 1.59 ***	n = 20
Plasma Renin Concentration (ng Ang I $ml^{-1}hr^{-1}$)	102.4 ± 14.1	n = 25	54.3 ± 14.3 **	n = 17	33.7 ± 4.7 ***	n = 18
Plasma Renin Substrate (ng Ang I ml^{-1})	230 ± 16	n = 4	424 ± 69 ***	n = 8	610 ± 86 ***	n = 18
Renal Renin Concentration (ng Ang I $mg^{-1}hr^{-1}$)	10,222 ± 1,060	n = 25	6,473 ± 1,368 ***	n = 18	5,544 ± 770 ***	n = 21
Packed Cell Volume (%)	36.7 ± 0.6	n = 30	33.0 ± 1.4	n = 17	32.5 ± 0.6	n = 15

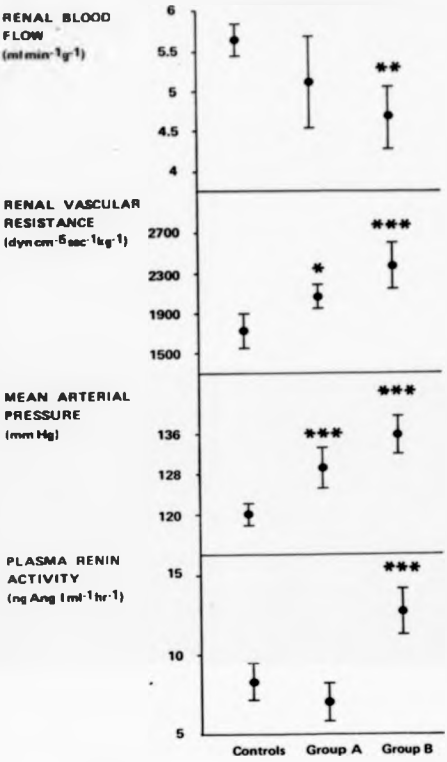


Fig.3.1 **The Effects of Oestrone Acetate on Renal Haemodynamics, Mean Arterial Pressure and Plasma Renin Activity. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, Significantly Different from Controls.**

Group A received 5mg and Group B 10mg of Oestrone Acetate.

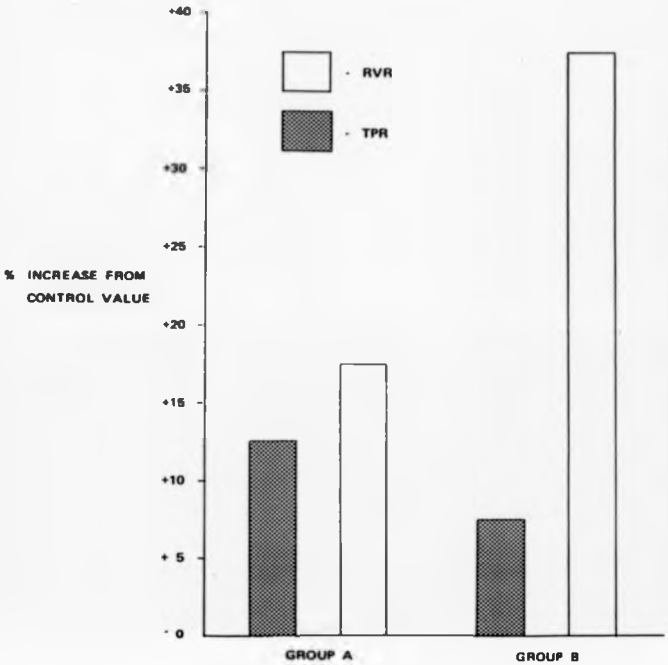


Fig. 3.2 The Effect of Oestrone Acetate on Peripheral and Renal Vascular Resistance. Data are Expressed as a Ratio of Measurements Made on Oestogen Treated compared to Control Values.

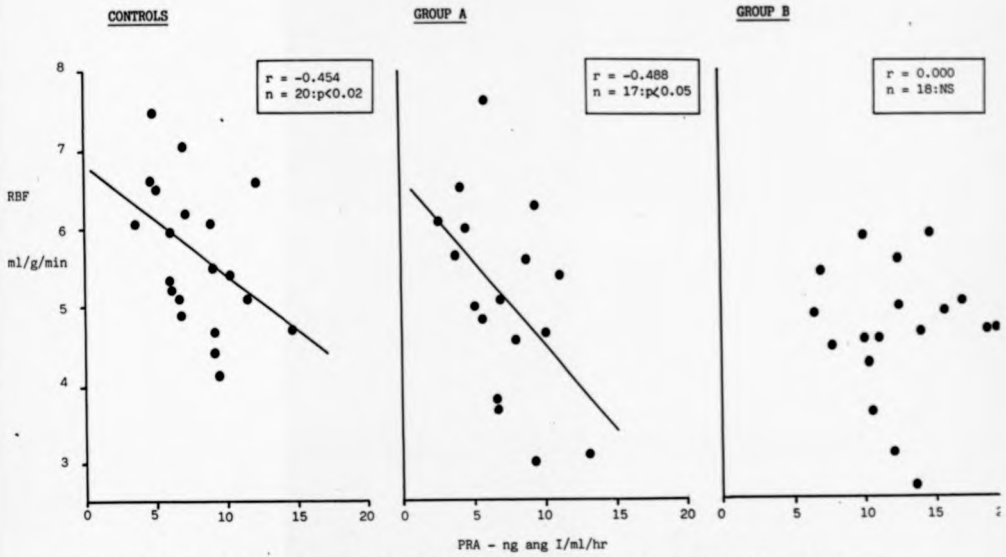


Figure 3.3 Correlations Between Renal Blood Flow and Plasma Renin Activity for the Control and Oestrogen Treated Groups of Rats. Legend as described in Figure 3.1. r = Correlation coefficient

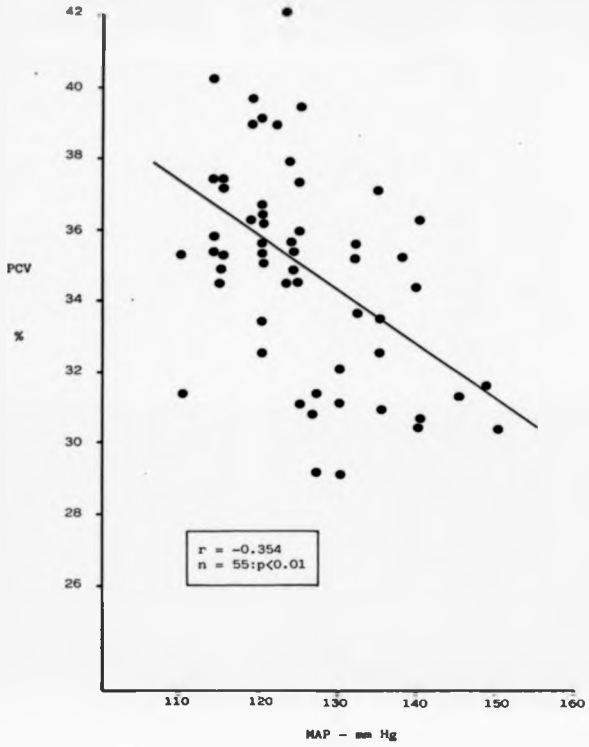


Figure 3.4 Correlation Between Packed Cell Volume and Mean Arterial Pressure. Data from all experimental animals has been utilised.

cell volume. Whereas both oestrogen regimes resulted in a fall in plasma and renal renin concentrations and a rise in plasma renin substrate concentration, an increase in plasma renin activity was only noted with the higher dose. A loss in body weight was noted in both groups of oestrogen treated rats.

Previous reports of the effect of oestrogen on renal haemodynamics have indicated that renal blood flow either remains unchanged (81-83) or increases slightly (84); a decrease in renal blood flow with oestrogen having not been previously reported. This disparity between previous reports and the present finding may well be due to differences in the nature of the oestrogen preparation used, the schedule of administration employed, or the species involved.

The fact that the smaller rise in mean arterial pressure (seen in animals receiving 5 mg oestrogen) took place without a change in renal blood flow, suggests that the oestrogen-induced fall in renal blood flow took place as the result of an antecedent rise in blood pressure. The lack of any relationship between these two haemodynamic variables is not, however, consistent with this chronology of events. This finding, coupled with the observation that oestrogen-induced increases in renal vascular resistance were much greater than changes in total peripheral resistance suggests either that the reactivity of the intrarenal vasculature -to humoral or neurogenic factors - is different from that of the systemic circulation in this particular animal model, or that the mechanisms responsible for the change in RVR were predominantly intrarenal. Both could be mediated by either a direct or indirect influence of the steroid. Although the suggestion of a direct intrarenal effect of oestrogen may be attractive in the knowledge that specific

oestrogen receptors are known to be present in the kidney (85), their distribution does not include vascular tissue, thus making it unlikely that oestrogen has a direct effect on renal vasculature. On the other hand, there is abundant evidence to support the view that oestrogens may influence intrarenal vascular resistance through indirect pathways. This includes the observations that oestrogens are known to activate the renin-angiotensin system (40), to increase the number of smooth muscle angiotensin II receptors (47), to enhance smooth muscle sensitivity to catecholamines (48) and vasopressin (49), to exert a variable effect on vascular prostaglandin activity (44-46) and to influence the kallikrein-kinin system (42,43).

The finding of a correlation between RBF and PRA in control and group A animals is consistent with the view that the RAS plays a role in the modulation of renal haemodynamics under conditions of normal RBF. However, the lack of any such relationship in animals with a reduced RBF makes it doubtful that the increase in PRA (and therefore, probably circulating angiotensin II) noted in these animals played a major role in determining the rise in RVR. This conclusion requires further consideration if one accepts that the concentration of renin or angiotensin II present in the systemic circulation may be a poor index of that present in the peripheral arterioles (86,87). One might therefore speculate, that under the influence of oestrogen, an excessive accumulation of either renin or renin substrate occurs in renal resistance vessels, leading to an enhanced generation of Ang II locally. The fall in RRC recorded in oestrogen treated animals does not necessarily detract from this hypothesis, since RRC chiefly reflects renin production and/or storage by the juxtaglomerular apparatus and is not

therefore a valid reflection of its concentration in the renal vasculature itself.

Oestrogen may also have the capacity to modulate the intrarenal action of angiotensin II, by changing the number and/or affinity of intrarenal vascular angiotensin II receptors. Support for this theory may be derived from the observation that oestrogen sensitises the myometrium to the effect of angiotensin II through an increase in the number of angiotensin II receptors (47). Recent studies in our own laboratory, which have demonstrated that oestrogen increases the number of angiotensin II receptors in the glomeruli of normotensive rats (A. Messenger: personal com)give further support to this idea.

The possibility that the oestrogen-induced fall in RBF might also have resulted from the influence of this steroid on either, other vasoactive compounds involved in the modulation of renal haemodynamics, such as prostaglandins (44-46) or kinins (42,43) or a stabilisation effect on cell membranes (88) cannot be excluded by this study.

Previous reports on the effect of oestrogen on blood pressure in the rat (89,90) are in agreement with the increases in mean arterial pressure seen in this study. The level of blood pressure in the body is determined by the product of cardiac output and total peripheral resistance. The latter two variables in turn, are chiefly set by respective changes in extracellular fluid volume and the balance of vasoactive compounds. These 'volume' and 'vasoconstrictor' components usually move in opposite directions to maintain a normal level of blood pressure. Excessive increases in either cardiac output or total peripheral resistance are the respective hall-marks of volume or vasoconstrictor mediated hypertension. However, the lack of any

marked change in either cardiac output or total peripheral resistance, suggests the oestrogen-induced increase in mean arterial pressure encountered in this study involved a combination of both volume and vasoconstrictor factors.

Given that a reduction in packed cell volume reflects an enlargement in plasma volume (77), the fall in packed cell volume noted in both oestrogen treated groups of rats is consistant with the involvement of a prominent volume component in the hypertension encountered in these animals. Powerful support for this hypothesis may be derived from the present observation of a strong reciprocal relationship between mean arterial pressure and the packed cell volume.

One might have anticipated that the oestrogen-induced expansion of plasma volume, described above, would have resulted in a decrease in plasma renin activity, rather that the unchanged and increased values noted in animals receiving 5 and 10 mg of oestrogen respectively. This apparently inconsistant finding may be explained by the fact that oestrogen increases the hepatic biosynthesis of renin substrate (39) - as confirmed by this study. This increase would result in firstly, a rise in circulating angiotensin II (detected as an increased plasma renin activity) and secondly, a fall in renin secretion (detected as a decreased plasma and renal renin concentration) by a direct negative feedback pathway. The overall effect of these changes in the renin-angiotensin system would be to nullify any compensatory fall in total peripheral resistance, which might have been expected to occur in response to an increase in plasma volume.

On the basis of the above findings, it seems likely that the mechanisms leading to the establishment of an

oestrogen-induced increase in blood pressure, as noted in this study, involve a combination of expanded plasma volume, coupled with a renin-angiotensin activity which is inappropriately high in relation to the volume status of the animal. Moreover, an increased plasma volume appears to be the primary event responsible for the establishment of the increased blood pressure, which may be exacerbated by activation of the renin-angiotensin system. Support for this hypothesis may be derived from the observation that whereas mean arterial pressure was inversely related to packed cell volume, it showed no relationship to plasma renin activity. Further, an increased plasma renin activity was only noted in animals exhibiting the larger rise in MAP.

Extracellular fluid volume expansion thus appears to be the primary event leading to the establishment of the elevated mean arterial pressures seen in this study. This expansion may be initiated by sodium retention. The antinatriuretic properties of oestrogen have long been recognised (91-93), and may account for the oedema seen in some women, both prior to menstruation (94,95) and during late pregnancy (96); circumstances when blood oestrogen levels are elevated. The site of this property is predominantly intrarenal (though not necessarily mediated through the adrenal glands) (97), although extrarenal sites have also been identified (98).

The involvement of the renin-angiotensin system in oestrogen-associated hypertension is demonstrated by the report of Stubbs et al (99). In this study, the competative angiotensin II antagonist (Sar-1Ala-8) angiotensin II was infused into rats with elevated blood pressure levels through oral contraceptive pretreatment (Enovid), and a return of blood pressure to the

pretreatment levels noted.

In conclusion, oestrogen has been shown to affect marked changes in renal and systemic haemodynamics, the renin-angiotensin system and plasma volume status in the normotensive rat. A reduction in renal blood flow occurred independently of a rise in mean arterial pressure. This reduction in renal blood flow could not be accounted for by a change in cardiac output, but was due to an increased renal vascular resistance in the presence of an elevated plasma renin activity. This observation suggests that angiotensin II-mediated vasoconstriction of the renal vasculature was responsible for the reduced renal perfusion described above. The rise in mean arterial pressure appeared to be principally determined by salt and water retention, in conjunction with a plasma renin activity which was inappropriately high in relation to plasma volume status.

Chapter 4

INACTIVE RENIN AND OESTROGEN TREATMENT

4.1 INTRODUCTION

Renin is an acid protease, and as such might be expected to exist in an enzymatically inactive precursor form. Indeed, other members of the same family are synthesised as proenzymes; for example - pepsin. Recently, an intracellular prorenin has been identified (100, 101) and its amino acid sequence elucidated (102, 103). This large molecular weight prorenin is converted to active renin intracellularly. Plasma and other biological fluids are also known to contain a form of renin with negligible enzymatic activity. The origin of the experimental data describing the existance of this 'inactive' renin can be traced to the work of Lumbers in 1971. She showed that amniotic fluid dialysed to a pH of 3.3, and then back to 7.4, showed a higher renin concentration than amniotic fluid dialysed to pH 4 or above. She suggested that this effect was due to the activation of an inactive form of renin by acid (104).

Inactive renin may be activated in vitro using a variety of procedures. These procedures involve acidification, cold treatment or the use of proteolytic enzymes. Acid activation of inactive renin has been mentioned above. If proteolytic enzymes are added to plasma, amniotic fluid or kidney extracts, and the proteolytic activity arrested before the mixture is assayed for renin, an increase in renin concentration may occur. Trypsin (105), Cathepsin D (106), plasmin (107), glandular kallikrein (108) and plasma kallikrein (109) are all effective as activators of inactive renin. The exposure of human plasma to a temperature of $-5^{o}C$ for 4 days results in an increase in renin enzymatic activity (110), but to a lesser extent than treatment with either acid or trypsin.

The biochemical nature of inactive renin has been the

subject of much speculation (111, 112) but remains as yet, largely unknown. The most likely explanation is that inactive renin is a proenzyme precursor of active renin. This being so, an increase in active plasma renin concentration would be associated with a decrease in inactive plasma renin concentration. Although the findings of a number of studies support this claim (113, 114), the evidence is by no means conclusive (115). Alternatively, inactive renin might be an inhibitor-bound form of active renin. The inhibitor could be present in the same cell as active renin, or could be synthesised elsewhere, to bind with active renin after active renin has been released.

The physiological importance of inactive renin to the organism is obscure. The demonstration of an event in a test-tube does not mean that the event occurs in vivo; much less that it is of biological significance to the organism. Nevertheless, the reports that both kallikrein (108, 109) and plasmin (107) can activate inactive renin suggests that this activation may occur in vivo. This being so, the heterogeneity of the renin molecule is of significant relevance to this investigation.

Compared to other species, reports concerning an inactive form of renin in the rat are few in number and contradictory in nature. Moreover, there have been no reports of the effect of oestrogen on inactive renin in the rat. A solitary report by Hedlin, et al (116) showed that circulating inactive renin levels were normal in women ingesting oral contraceptives.

From the preceding chapter, it will be remembered that oestrogen treatment had a marked effect on renal haemodynamics. An increased renin-angiotensin activity (active renin) could not

satisfactorily account for this effect. It is tempting to speculate therefore, that conversion of inactive to active renin - perhaps mediated intrarenally by kallikrein- may be an important contributory mechanism to the development and maintenance of an increased renal vascular resistance during oestrogen treatment.

The objectives of this study therefore, were to clarify the uncertainty surrounding the existence of an inactive form of renin - of both plasma and renal origin - in the rat, and if appropriate, to assess the influence of oestrogen treatment on inactive renin. By using specimens obtained from the oestrogen treated rats described in the preceding chapter, it may be possible to ascertain the pathophysiological significance of the heterogenous renin molecule to haemodynamic function, in the oestrogen treated rat.

4.2 METHODS

The plasma and renal cortex tissue used for this study were obtained from the control and oestrone acetate treated groups A (5 mg oestrogen) and B (10 mg oestrogen) described in the preceding chapter. The materials and methods used to measure both plasma renin activity and renal renin concentration have been fully described in chapters 2 and 3. The incorporation of trypsin treatment and acidification into Stage 1 (Generation of angiotensin I) of these methods, to facilitate the measurement of inactive plasma and renal renin respectively, is described below. These procedures allow the simultaneous measurement of both inactive and active (plasma renin activity and renal renin concentration) renin. Data relating to plasma renin activity measurements have been previously presented in chapter 3.

4.2.1 The Effect of Trypsin Treatment on Plasma Renin Activity

The presence of a trypsin activable form of renin in rat plasma has been described (117, 118). The following procedure, based on these reports, was adopted to investigate the effect of trypsin treatment on plasma renin activity. Immediately prior to use, trypsin (type 1 X, Sigma, UK) and soya bean trypsin inhibitor (Type 1S, Sigma) were dissolved in incubation buffer pH 6.5, to give stock solutions of 60 and 66 mg per ml respectively.

After the addition of 5 µl inhibitor cocktail during stage 1 (See p 36), 25 µl trypsin solution was added to the incubate, to give a final concentration of trypsin in plasma of 6 mg per ml. The mixture was incubated at 4°C for 4 minutes. The action of trypsin was terminated by the addition of 25 µl soya bean trypsin inhibitor solution (6.6 mg per ml plasma). 50 µl incubation buffer was added to control plasmas. The concentrations of trypsin and soya bean trypsin inhibitor used in this study have been shown to effect maximal activation of inactive plasma renin with minimal inhibition and degradation of renin (117). Samples were incubated and assayed for renin activity as previously described.

The angiotensin I generated from trypsin treated and control samples was termed total renin activity (TRA) and plasma renin activity (PRA) respectively. Inactive renin was defined as the difference between these two measurements (TRA - PRA).

4.2.2 The Effect of Acidification on Renal Renin Concentration

The existence of an acid activable from of renin in

human kidney has been described by Haueh, et al (119). The following procedure, based on this report, was used to investigate the effect of acidification on renal renin concentration.

After homogenisation of the cortex sample and subsequent centrifugation of the homogenate, a 1 ml portion of homogenate supernatant was placed in dialysis tubing (Scientific Industries, UK). The supernatant was dialysed against approximately 20 volumes of 0.05M glycine buffer pH 3.3, for 20 hours, and then redialysed to pH 7.5 against incubation buffer for a further 20 hours. Another 1 ml portion of supernatant was dialysed against incubation buffer pH 7.5 for 40 hours and used as the dialysed control. Dialysis took place at 4°C: both acid and neutral buffers contained 0.1M sodium chloride.

Portions of undialysed and dialysed supernatant were subsequently assayed for protein content and renin concentration respectively. The angiotensin I generated from acid dialysed and control samples was termed total renal renin concentration and renal renin concentration respectively. As for plasma, inactive renin was defined as the difference between these two sets of measurements - TRC - RRC.

4.3 RESULTS

The injection of vehicle alone did not alter any of the parameters under study (Table 4.1). As in chapter 3 therefore, data from vehicle injected animals has been pooled and referred to as the control group, against which oestrogen treated animals have been compared (Table 4.2 and 4.3).

Effect of Trypsin Treatment on Plasma Renin Activity (Table 4.4 and Fig 4.1)

Trypsin treatment resulted in a significant increase in plasma renin activity from 8.35 ± 1.22 to 8.90 ± 1.13 ng ang I ml^{-1} hr^{-1} ($p < 0.05$) in controls. Whilst trypsin treatment also led to an increase in plasma renin activity in both group A, from 6.97 ± 1.39 to 10.91 ± 1.39 ng ang I ml^{-1} hr^{-1} ($p < 0.001$) and group B, from 12.67 ± 1.59 to 13.68 ± 1.02 ng ang I ml^{-1} hr^{-1} (NS), the change in plasma renin activity noted in group B was not statistically significant.

Effect of Acidification on Renal Renin Concentration (Table 4.4 and fig 4.2)

Acidification resulted in a significant increase in renal renin concentration from 10,632 ± 1,082 to 11,606 ± 1,268 ng ang I mg^{-1} hr^{-1} ($p < 0.001$) in controls. Acid dialysis also led to significant increases in renal renin concentration in oestrogen treated animals. Renal renin concentration rose from 7,025 ± 1,288 to 7,914 ± 1,427 ng ang I mg^{-1} hr^{-1} ($p < 0.01$) in group A, and from 5,934 ± 819 to 6,372 ± 833 ng ang I mg^{-1} hr^{-1} ($p < 0.01$) in group B.

Effect of Oestrogen on Inactive Renin

Inactive plasma renin concentration (total renin activity - plasma renin activity) was 0.72:0 - 2.17 ng ang I ml^{-1} hr^{-1} in controls, which comprised 9.67 ± 3.57 % of total renin activity. The administration of 5mg oestrogen caused a significant increase in the amount of plasma inactive renin to 3.97 ± 1.08 ng ang I m_l^{-1} hr^{-1} ($p < 0.001$) or 36.34 ± 11.00 % of total renin activity. No significant amount of inactive renin was detected in the plasma of rats who received 10 mg oestrogen.

Inactive renal renin concentration (total renin concentration - renal renin concentration) was 986:0 - 3,488 ng ang I ml^{-1} hr^{-1} or 7.87 $\pm$ 2.20% of total renin concentration, in controls. Oestrogen treatment did not alter these values (820:0 - 2,938 and 500 $\pm$ 206 ng ang I ml^{-1} hr^{-1} , or 12.00 $\pm$ 4.08 and 8.00 $\pm$ 3.37 % of total renin concentration in groups A and B respectively).

Relationship Between Inactive and Active Renin (Fig 4.3)

Using data from all experimental animals, a significant inverse correlation was noted between inactive and active plasma renin ($p<0.001$). Further, the regression of inactive renin on active renin was found to be significant ($p<0.001$). No such relationship was noted between inactive and active renal renin.

4.4 DISCUSSION

The findings from this study have demonstrated the presence of a trypsin-activable form of inactive renin in rat plasma. Whilst the administration of 5 mg oestrogen resulted in a significant increase in this inactive renin, no inactive renin was detected in the plasma of rats which received 10 mg oestrogen. An acid-activable form of inactive renin was detected in rat renal extract. This inactive renin was not affected by oestrogen treatment. An inverse relationship was noted between inactive and active plasma renin, with changes in inactive renin being dependant upon changes in active renin.

The existence of an inactive form of renin in human plasma is well established, and accounts for greater than 50% of total renin (120). In the rat however, controversy surrounds the existence of an inactive form of plasma renin. Osmond and Cooper (121) were unable to detect any inactive renin, using methodology which had successfully detected this renin in several other

TABLE 4.1 Effect of Vehicle (ethyl oleate) Injection on Inactive and Active Renin

Data are presented as either a mean ± 95% confidence limits, or a median and range. In the test column an F value indicates the use of a one way ANOVA: a Z value, the Wilcoxon rank sum test for two groups, and a H value, the Kruskal-Wallis test for three groups. NS = non significant.

PARAMETER	NON TREATED	CONTROL FOR GROUP A	CONTROL FOR GROUP B	TEST
Plasma Renin Activity (ng Ang I ml^{-1} hr^{-1})	7.30 ± 0.86 n=5	6.51 ± 1.20 n=8	9.21 ± 1.55 n=17	F = 2.86 NS
Total Renin Activity (ng Ang I ml^{-1} hr^{-1})	-	7.39 ± 0.94 n=8	9.61 ± 1.51 n=17	F = 3.58 NS
Inactive Plasma Renin (ng Ang I ml^{-1} hr^{-1})	-	0.92 ± 0.43 n=8	0.34: 0-2.17 n=17	Z =-1.11 NS
Inactive Plasma Renin (% Total Renin Activity)	-	13.06 ± 6.31 n=8	4.21: 0-25.56 n=17	Z =-1.81 NS
Renal Renin Concentration (ng Ang I mg^{-1} hr^{-1})	13,006 ± 3,042 n=4	9,012 ± 1,605 n=7	11,255 ± 1,107 n=18	F = 3.75 NS
Total Renin Concentration (ng Ang I mg^{-1} hr^{-1})	14,530 ± 4,318 n=4	9,742 ± 1,793 n=7	12,082 ± 1,321 n=18	F = 3.40 NS
Inactive Renal Renin (ng AngI mg^{-1} hr^{-1})	1,525 ± 1,421 n=4	766 ± 508 n=7	966: 0-2,897 n=18	H = 2.33 NS
Inactive Renal Renin (% Total Renin Concentration)	9.11 ± 7.08 n=4	7.68 ± 4.47 n=7	6.54: 0-17.30 n=18	H = 1.69 NS

TABLE 4.2 Data Relating to the Renin-Angiotensin System in the Oestrone Acetate Treated and Control Groups of Rats

Legend as described in Table 4.1. Group A received 5 mg of oestrogen and Group B 10 mg. *, $p<0.05$: ***, $p<0.01$: ***, $p<0.001$ - significantly different from controls.

PARAMETER	CONTROLS	GROUP A	GROUP B
Plasma Renin Activity (ng Ang I ml^{-1} hr^{-1})	8.35 ± 1.22 n=25	6.97 ± 1.39 n=18	*** 12.67 ± 1.59 n=20
Total Renin Activity (ng Ang I ml^{-1} hr^{-1})	8.90 ± 1.13 n=25	* 10.91 ± 1.39 n=18	*** 13.68 ± 1.02 n=20
Inactive Plasma Renin (ng Ang I ml^{-1} hr^{-1})	0.72: 0-2.17 n=25	*** 3.97 ± 1.08 n=18	1.52 ± 0.86 n=20
Inactive Plasma Renin (% Total Renin Activity)	9.67 ± 3.57 n=25	*** 36.34 ± 9.11 n=18	5.19: 0-50.42 n=20
Renal Renin Concentration (ng Ang I mg^{-1} hr^{-1})	10,632 ± 1,082 n=30	*** 7,025 ± 1,288 n=18	*** 5,934 ± 819 n=21
Total Renin Concentration (ng Ang I mg^{-1} hr^{-1})	11,605 ± 1,268 n=30	*** 7,914 ± 1,427 n=18	*** 6,372 ± 833 n=21
Inactive Renal Renin (ng Ang I mg^{-1} hr^{-1})	986: 0-3,488 n=30	820: 0-2,938 n=18	500 ± 206 n=21
Inactive Renal Renin (% Total Renin Concentration)	7.87 ± 2.20 n=30	12.00 ± 4.08 n=18	8.00 ± 3.37 n=21

TABLE 4.3 Statistical Analysis of the Effects of Oestrogen Treatment on the Renin-Angiotensin System

Legend as described in Table 4.1

PARAMETER	CONTROLS v. GROUP A	CONTROLS v. GROUP B	GROUP A v. GROUP B
Plasma Renin Activity	F = 2.11 NS	F = 18.72 ***	F = 27.76 ***
Total Renin Activity	F = 4.90 *	F = 35.98 ***	F = 10.04 **
Inactive Plasma Renin	Z = 4.12 ***	Z = 0.87 NS	Z = 3.22 ***
Inactive Plasma Renin - %	F = 35.52 ***	Z = 0.02 NS	Z = 3.65 ***
Renal Renin Concentration	F = 16.95 ***	F = 39.52 ***	F = 2.08 NS
Total Renin Concentration	F = 12.95 ***	F = 36.58 ***	F = 3.58 NS
Inactive Renal Renin	Z = -0.08 NS	Z = -1.43 NS	Z = 1.39 NS
Inactive Renal Renin - %	F = 3.65 NS	F = 0.00 NS	F = 1.51 NS

TABLE 4.4 Statistical Analysis of the Effect of Trypsin Treatment and Acidification on Plasma Renin Activity and Renal Renin concentration Respectively

Data have been analysed using a paired t-test. The level of significance of the test statistic t is represented in the test column.

	CONTROL GROUP (no treatment)	EXPERIMENTAL GROUP (trypsin treatment or acidification)	MEAN DIFFERENCE BETWEEN GROUPS	N	TEST
CONTROLS	Plasma Renin Activity	Total Renin Activity	- 0.55	25	T = -2.53 *
	Renal Renin Concentration	Total Renin Concentration	- 927	30	T = -5.03 ***
GROUP A	Plasma Renin Activity	Total Renin Activity	- 3.94	18	T = -6.99 ***
	Renal Renin Concentration	Total Renin Concentration	- 888	18	T = -3.24 **
GROUP B	Plasma Renin Activity	Total Renin Activity	- 1.01	20	T = -1.72 NS
	Renal Renin Concentration	Total Renin Concentration	- 438	21	T = -3.52 ***

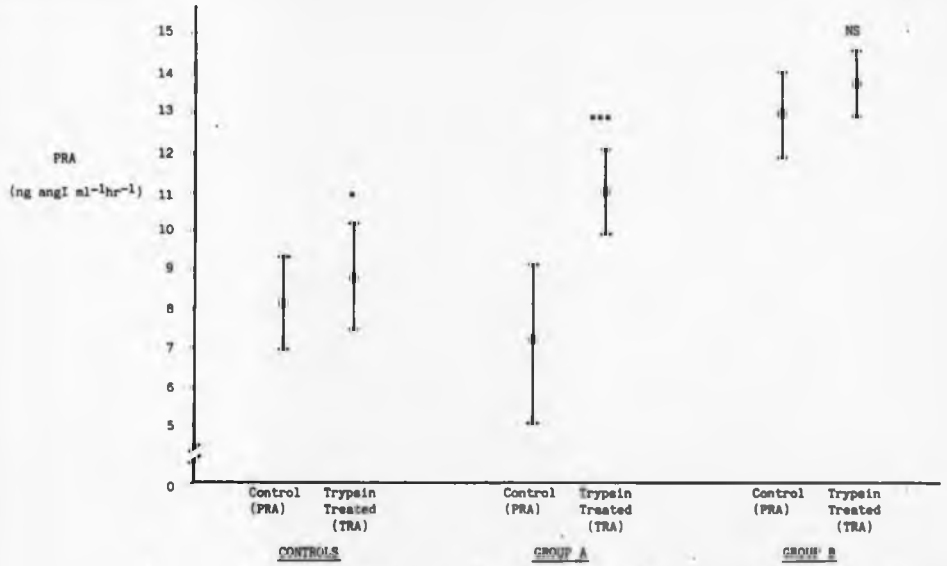


Figure 4.1 The Effect of Trypsin Treatment on the Plasma Renin Activity of Oestrogen Treated Rats and Their Controls

Group A received 5mg and Group B 10mg of Oestrone Acetate. Data was analysed using a paired t-test

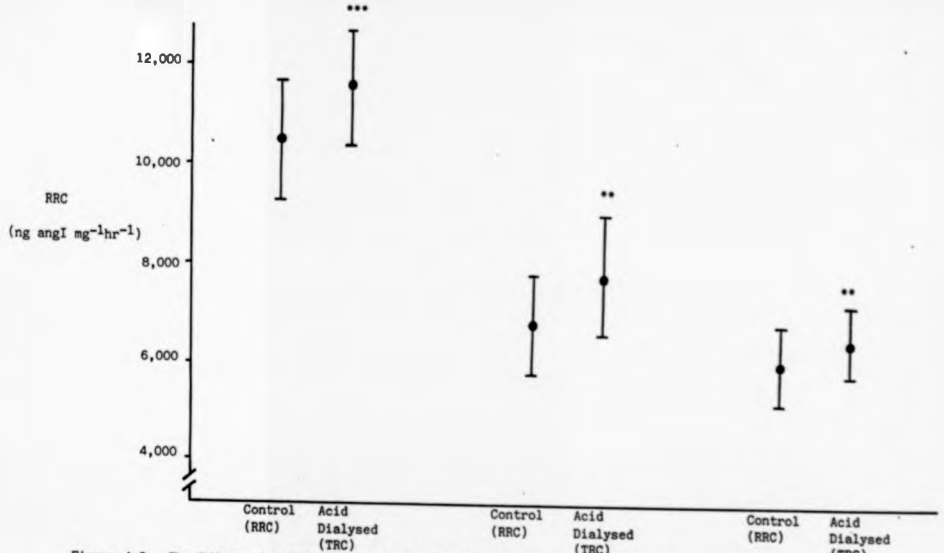


Figure 4.2 The Effect of Acidification on the Renal Renin Concentration of Oestrogen Treated Rats and Their Controls. Group A received 5mg and Group B 10mg of Oestrone Acetate. Data were analysed using a paired t-test.

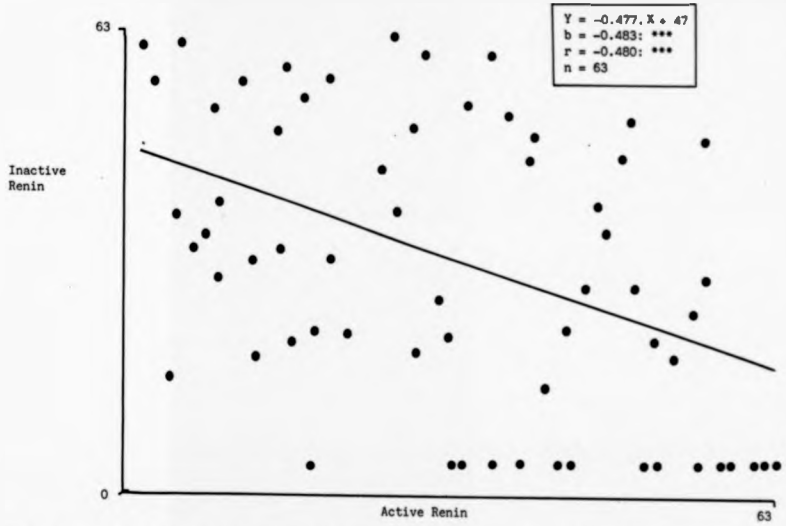


Figure 4.3 The Correlation and Regression of Inactive Plasma Renin on Active Plasma Renin

Data from all experimental animals has been used. As data was non-normally distributed, statistical analysis has been performed on ranked values. b - regression coefficient, r - correlation coefficient.

species. On the other hand, Vandongen, et al (122) detected inactive renin in plasma taken from anaesthetised animals, which accounted for 10% of total renin. Using unanaesthetised animals, Glorioso, et al (118) and Barrett, et al (117) also detected an inactive form of renin which comprised 40 and 85% of total renin respectively. The present finding of an inactive form of plasma renin which accounted for 8% of total renin, is concordant with the latter reports and closely matches the findings of Vandongen, et al.

If controversy surrounds the presence of an inactive form of renin in rat plasma, then the existence of an inactive renin of renal origin is even more uncertain. Using identical acidification procedures, Lauritsen, et al (123) could not detect inactive renal renin in the rat, but were able to detect inactive renin in porcine renal extracts. Nakane, et al (124) found that renin released from isolated perfused rat kidney was not activated by acidification. In contrast to these reports, Morris and Johnston (125) have reported the presence of an acid-activable form of inactive renin in rat renal extract. The present finding, that acidification increased the renal renin concentration of crude renal extract is in agreement with the latter report.

Data from several species including the rat, indicate that the kidney contains an inactive form of renin (119, 123, 125-127) which is the major source of the inactive material present in plasma (115,128). A recent study by Takii, et al (129) provides strong evidence to support the concept that inactive renin is a proenzyme precursor for renin. The reciprocal relationship which was noted between inactive and active renin in the present study is in agreement with this concept.

Parallel measurements of inactive renin from peripheral and

renal venous plasma suggest that activation of circulating inactive renin is a physiological event, occurring at an intrarenal site (119). The observation that urinary (renal) kallikrein is a potent activator of plasma inactive renin suggests that intrarenal kallikrein is the physiological activator of circulating inactive renin (108). Conversion of inactive to active plasma renin might therefore be an important physiological event, serving to regulate the amount of angiotensin II available to the renal circulation, as a supplemental mechanism to the process of renin release.

Oestrogens are known to stimulate the kallikrein-kinin system (42,43). They may achieve this either by a direct effect or through the renin-angiotensin axis (130, 131). The changes in plasma inactive renin noted in this study may reflect an influence of oestrogen on kallikrein-mediated conversion of inactive to active plasma renin. For example, an increase in kallikrein secretion may have enhanced the conversion of inactive to active plasma renin (thus depleting plasma inactive renin) in rats which received 10 mg oestrogen. The increased intrarenal generation of angiotensin II which would ensue, may have contributed to the maintenance of the increased renal vascular resistance which was noted in these animals.

In conclusion, this study has provided evidence which is consistent with the presence of an inactive form of renin in both the kidney and plasma of the rat. Evidence was also found to support the concept that inactive renin is a proenzyme precursor for renin. It has been suggested that oestrogen may influence kallikrein-mediated conversion of inactive to active renin. This effect might contribute to the maintenance of an increased renal vascular resistance in the oestrogen treated rat.

Chapter 5

THE EFFECTS OF ANGIOTENSIN II BLOCKADE ON RENAL AND SYSTEMIC HAEMODYNAMICS IN THE OESTROGEN TREATED NORMOTENSIVE RAT.

5.1 INTRODUCTION

The findings from Chapters 3 and 4 clearly show that oestrogen-induced changes in both renal and systemic haemodynamics in the rat were associated with marked alterations in the renin-angiotensin system. Whilst these findings suggest that this system is involved in implementing the haemodynamic changes, they provide no supportative evidence, and permit no precise conclusions as to the nature of the involvement.

The classical method of discovering the function of an endocrine system in various physiological and pathological states is to eliminate the source of the hormone. This approach is not feasible for studies involving the renin-angiotensin system. The juxtaglomerular apparatus is in intimate contact with the organ it exerts a major influence upon, the kidney. However, the recent advent of pharmacological compounds, which can interrupt the renin-angiotensin system at critical stages between renin secretion and the interaction of angiotensin II with its receptors, now allow temporary, reversible ablation of the renin-angiotensin system. The two most widely used classes of inhibitors of the renin-angiotensin system are angiotensin converting enzyme inhibitors and angiotensin II analogues. These pharmacological tools have been invaluable in defining the role of the renin-angiotensin system in both normal (146-148, 155) and abnormal (149,150,156) cardiovascular function. Their application, to discover the importance of angiotensin II to the maintenance of arterial pressure and renal blood flow in the salt-deplete state, has been mentioned during the course of this investigation.

Converting enzyme inhibitors, such as captopril, block the cleavage of angiotensin I, and thereby prevent the formation of

angiotensin II (151). These compounds also inhibit the degredation of kinins however, potentiating the vasodilatory property of these substances (152). Caution is therefore necessary when interpreting haemodynamic changes associated with the use of a converting enzyme inhibitor.

A number of angiotensin II analogues have been synthesised by making amino acid substitutions at each position in the sequence of the native hormone (153). The combined substitutions of sarcosine in position 1 - which confers long life and increased binding affinity, and alanine in position 8 - which confers antagonistic properties, has resulted in the synthesis of Sar 1, Ala 8 - angiotensin II, or Saralasin - the most commonly used analogue of angiotensin II.

Saralasin competes with angiotensin II for available receptor sites, and is a competitive antagonist. Like all of the peptide antagonists of angiotensin II synthesised to date, saralasin may also act as a partial agonist. This intrinsic activity can be found in tissues which are most sensitive to angiotensin II, such as the renal vasculature (154).

Angiotensin II analogues have been useful in clarifying the role of angiotensin II in both normal cardiovascular homeostasis (146-148) and abnormal cardiovascular function (149-150), in characterising angiotensin II receptors (154,157) and in providing a potential for the elucidation of the aetiology of human hypertension under some circumstances (149,158). Of particular interest to this study are the reports that saralasin affected a fall in arterial pressure, both in pregnant rats (159), and in rats receiving an oestrogen-containing oral contraceptive (160). These reports support the contention that an increased concentration of circulating angiotensin II is a causal factor in mediating

oestrogen-induced haemodynamic disturbances.

The objective of this study therefore, was to assess the contribution of angiotensin II to haemodynamic function in the oestrogen treated rat, through the use of the angiotensin II analogue, saralasin.

5.2 METHODS

Oestrone acetate in ethyl oleate (4 mg per ml) was used to give subcutaneous injections of 1 mg oestrogen per day for 10 days, to a group of adult female Sprague-Dawley rats (170 - 240 g). Animals were housed and fed as described in chapter 3. Control animals received a daily injection of vehicle for 10 days.

24-hours after their last injection of either vehicle or oestrogen, animals were prepared for experimental analysis as described in chapter 2. In addition, a third polythene catheter (outer diameter = 0.75 mm) was secured in the left femoral vein of oestrogen treated rats, to facilitate intravenous infusion.

In control animals, renal and systemic haemodynamics, plasma and renal renin and the packed cell volume were measured as described in chapter 2. In oestrogen treated animals, resting mean arterial pressure was measured using the carotid catheter. Animals were then infused with either saline (0.034 ml per min) or saralasin (Sarenin, 2 µg per min: Rohm, W. Germany) diluted in saline, for 15 minutes, using a Braun (W. Germany) 871104 continuous drive perfusor. This amount of saralasin was found to inhibit the pressor response to a 10ng bolus of angiotensin II injected intravenously into oestrogen treated rats (160). At the end of the infusion period mean arterial pressure was again measured. The carotid catheter was advanced into the left ventricle, and cardiac output, renal blood flow and

vascular resistance determined as described in Chapter 2. Plasma and renal renin and the packed cell volume were also measured as described in this chapter.

Statistics

The effect of infusions, on both mean arterial pressure and heart rate, have been analysed by comparing those values obtained prior to infusion to those values noted immediately after the infusion of either saline or saralasin. Data were analysed using a paired t-test. For the remaining parameters, the influence of saralasin was assessed by comparing data obtained from animals infused with saline to those obtained from animals infused with saralasin - using a one way analysis of variance.

5.3 RESULTS

As in preceding chapters, the injection of vehicle did not affect any of the parameters under study (Table 5.1). Data from these animals was therefore combined for the purpose of comparison with oestrogen treated animals.

The Effects of Saralasin on Haemodynamics and the Renin-Angiotensin System (Table 5.2 and Figures 5.1 and 5.2)

Mean Arterial Pressure and Heart Rate

Prior to the infusion of saline, mean arterial pressure and heart rate were 126 ± 8 mm Hg and 355 ± 22 beats per min (n = 9). at the end of the infusion period these values remained unchanged at 126 ± 9.5 mm Hg and 365 ± 13 beats per min.

The infusion of saralasin had no significant effect on either mean arterial pressure (136 ± 6 v. 128 ± 8 mm Hg) or heart rate (384 ± 24 v. 390 ± 14 beats per min. n = 10).

Total Peripheral Resistance and Cardiac Output

Saralasin infusion resulted in an increase in total peripheral resistance from 32,301 ± 4,322 to 48, 779 ± 12,934 dyn cm^{-5} sec^{-1} kg^{-1} ($p< 0.05$), and a decrease in cardiac output from 321 ± 33 to 240 ± 53 ml min^{-1} kg^{-1} ($p<0.05$).

Renal Haemodynamics

Although saralasin reduced renal blood flow from 5.31 ± 0.82 to 3.41 ± 1.49 ml min^{-1} g^{-1}, this change did not achieve statistical significance ($p< 0.1 >0.05$). An increase in renal vascular resistance from 2,054 ± 502 to 4,504 ± 1,750 dyn cm^{-5} sec^{-1} kg^{-1} ($p< 0.05$) was noted after saralasin infusion.

The Renin-Angiotensin System

An increase in both plasma renin activity from 11.79 ± 3.31 to 79.83 ± 28.04 ng Ang I ml^{-1} hr^{-1} ($p<0.001$), and plasma renin concentration from 21.3 ±4.5 to 197.6 ± 82.9 ng Ang I ml^{-1} hr^{-1} ($p< 0.001$), was noted after saralasin infusion.

Renal renin concentation fell from 6,237 ± 998 to 3,076 ± 688 ng Ang I mg^{-1} hr^{-1} ($p<0.001$) after saralasin infusion.

Packed Cell Volume

Saralasin increased the packed cell volume from 29.9 ± 1.6 to 33.8 ± 2.2 % ($p< 0.05$). This latter value was significantly smaller than the value recorded for non-oestrogen treated rats ($F = 10.98$, $p< 0.01$).

5.4 DISCUSSION

The findings from this study have shown that in the oestrogen treated rat, an intravenous infusion of saralasin resulted in a 119% increase in renal vascular resistance, and a 51% increase in peripheral vascular resistance. Both plasma renin activity and concentration increased markedly after

TABLE 5.1. Effect of Vehicle (ethyl oleate) Injection on Haemodynamics, The Renin-Angiotensin System and Packed Cell Volume.

Data are presented as either a mean ± 95% C.L., or a median and range. In the test column an F value indicates the use of a one way ANOVA, and a Z value the use of the Wilcoxon Rank Sum test. NS = not significant

PARAMETER	NON TREATED	VEHICLE ~ 0.25 ml per day for 10 days	TEST
Mean Arterial Pressure mm Hg	117±4 n=9	124; 114-140 n=10	Z=-1.10 NS
Heart Rate beats per min	397±20 n=5	388±10 n=10	F= 0.86 NS
Cardiac Output ml min^{-1} kg^{-1}	240±25 n=9	236±27 n=10	F= 0.06 NS
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.57±0.04 n=5	0.61±0.08 n=10	F= 0.49 NS
Total Peripheral Resistance dyn cm^{-5} sec^{-1} kg^{-1}	39,890±4,416 n=9	42,419±4,777 n=10	F= 0.57 NS
Renal Blood Flow ml min^{-1} g^{-1}	5.35±0.82 n=9	5.97±0.65 n=10	F= 1.37 NS
Renal Vascular Resistance dyn cm^{-5} sec^{-1} kg^{-1}	1,743 1,315-3,154 n=9	1,667±176 n=10	Z= 0.24 NS
Plasma Renin Activity ng Ang I ml^{-1} hr^{-1}	7.88±0.69 n=5	8.90±2.06 n=9	F= 0.48 NS
Plasma Renin Concentration ng Ang I ml^{-1} hr^{-1}	97.90±26.07 n=5	109.6±32.3 n=9	F= 0.23 NS
Renal Renin Concentration ng Ang I mg^{-1} hr^{-1}	11,192±2,435 n=4	9,590±1,011 n=10	F= 0.04 NS
Packed Cell Volume %	37.9±1.6 n=5	37.5±1.6 n=10	F= 0.09 NS

TABLE 5.2. Effect of Saralasin Infusion (iv - 2 ng per min for 15 min) on Haemodynamics, the Renin-Angiotensin System and the Packed Cell Volume in Rats Given 1 mg Deoxycorticosterone Acetate per day for 10 days.

Legend as described in Table 5.1. Values for mean arterial pressure and heart rate are given for both before (B) and after (A) infusion.

PARAMETER	SALINE		SARALASIN		TEST
Mean Arterial Pressure mm Hg B A	126±8 126±9.5	n= 9	134±6 128±8	n=10	-
Heart Rate beats per min B A	355±22 365±13	n= 9	384±24 390±14	n=10	-
Cardiac Output ml min^{-1} kg^{-1}	321±33	n= 8	240±53	n=10	F= 5.82 *
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.93±0.10	n= 8	0.63±0.14	n=10	F= 9.45 *
Total Peripheral Resistance dyn cm^{-5} $sec^{-1}kg^{-1}$	32,301±4,322	n= 8	48,779±12,934	n=10	F= 4.61 *
Renal Blood Flow ml min^{-1} g^{-1}	5.31±0.82	n= 8	3.41±1.49	n=10	F= 4.13 NS
Renal Vascular Resistance dyn cm^{-5} sec^{-1} kg^{-1}	2,054±502	n= 8	4,504±1,750	n=10	F= 5.66 *
Plasma Renin Activity ng Ang I ml^{-1} hr^{-1}	11.79±3.31	n=10	79.83±28.04	n=10	F=21.95 ***
Plasma Renin Concentration ng Ang I ml^{-1} hr^{-1}	21.3±4.5	n=10	197.6±82.9	n=10	F=17.31 ***
Renal Renin Concentration ng Ang I mg^{-1} hr^{-1}	6,227±998	n=10	3,076±688	n=10	F=26.09 ***
Packed Cell Volume %	29.9±1.6	n=10	33.8±2.2	n= 9	F= 7.23 *

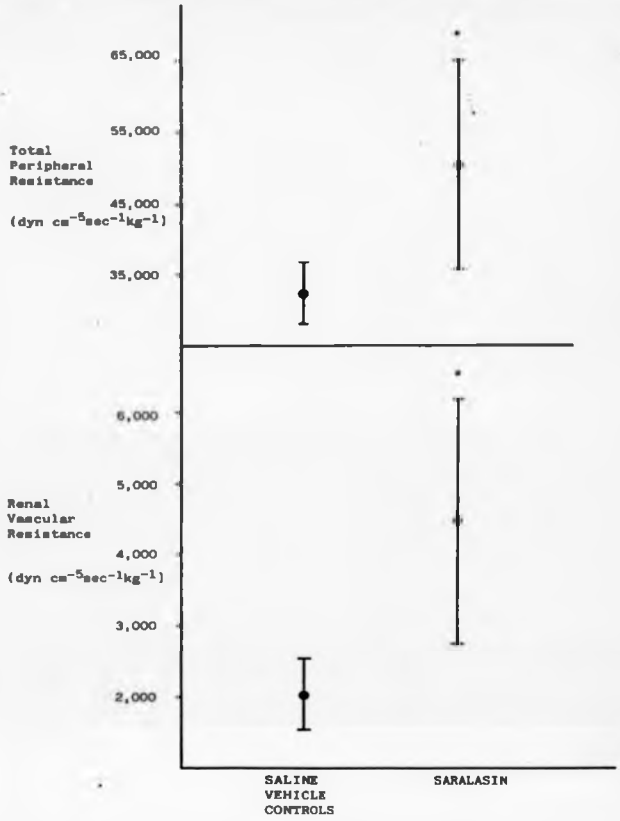


Figure 5.1 The Effects of Saralasin (i.v. -2µg per min. for 15 min.) on Total Peripheral Resistance and Renal Vascular Resistance in Oestrogen Treated Rats. Both groups of animals received 1mg of oestrone acetate per day for 10 days.

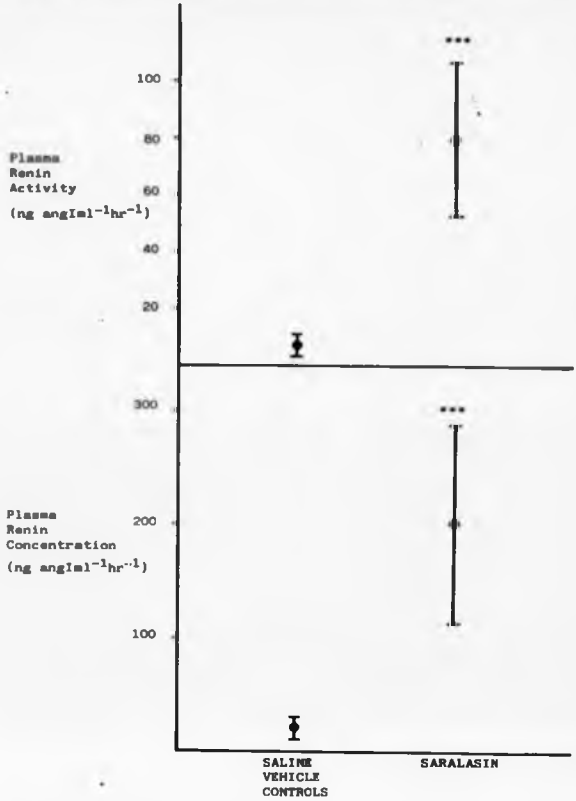


Figure 5.2 The Effects of Saralasin (i.v. -2μg per min. for 15min.) on the Plasma Renin Activity and Concentration of Oestrogen Treated Rats. Both groups of animals received 1mg of oestrone acetate per day for 10 days.

saralasin infusion.

In the normal, anaesthetized rat, saralasin acts as an antagonist, but has no effect on blood pressure, cardiac output, vascular resistance or renal blood flow (148,161). Data from the present study indicates that saralasin exhibits agonistic-like activity in the oestrogen treated rat, as evidenced by an increase in vascular resistance after infusion of the agent. This activity is a consequence of the structural similarities between saralasin and angiotensin II. Thus, whilst the analogue has affinity for angiotensin II receptors, it also exhibits intrinsic activity - albeit to a far lesser extent than the primary agonist. The agonistic property of saralasin is not apparent under normal circumstances (148,161), but may become pronounced in states characterised by sodium overload. Thus, in both metacorticoid- and deoxycorticosterone acetate (DOCA)-induced hypertension, saralasin not only failed to lower blood pressure, but elicited a pressor response (150,162). Likewise, in the salt-loaded rabbit, saralasin was found to induce a fall in renal blood flow (154). Throughout this investigation a reduction in the packed cell volume (that is, an increase in plasma volume) has been a hall-mark of oestrogen treatment. The findings of the present study thus agree with the reports which have shown that the agonistic activity of saralasin becomes prominant in states characterised by sodium overload.

Numerous studies have shown that variation in dietary sodium intake alters the concentration of circulating angiotensin II (135,136). Bellucci and Wilkes have shown that this manoeuvre also modulates the density of vascular angiotensin II receptors (163). A reduced concentration of circulating angiotensin II (consequent to sodium surfeit)

resulted in up-regulation of angiotensin II receptors, and vice-versa. By dissociating the effect of dietary sodium from that of circulating angiotensin II (by infusing angiotensin II into the circulation), these authors were able to show that angiotensin II itself directly influences receptor equilibrium. The pronounced intrinsic activity of saralasin, in the oestrogen treated rats described in this study, may therefore be the result of an increase in the density of vascular angiotensin II receptors in these sodium loaded animals.

The stimulatory effect of oestrogen on angiotensin II receptor number in both the myometrium (47) and the glomeruli of normotensive rats (A.Messenger:pers.comm) has been discussed previously. These observations suggest that oestrogen may increase the density of vascular angiotensin II receptors independently of circulating angiotensin II. Moreover, this effect of oestrogen would appear to have a major expression on renal vasculature, where it may, in the presence of an increased concentration of angiotensin II, lead to an increase in renal vascular resistance.

The blood pressure of oestrogen treated rats was not affected by the infusion of saralasin, and remained significantly elevated compared to control animals. This observation is in contrast to the finding of Stubbs, et al (160), where the same amount of saralasin was found to return the blood pressure of rats with oral contraceptive-induced hypertension to pretreatment levels. Although peripheral vascular resistance was not measured in this report, the fall in blood pressure was presumably due to a decrease in this variable - the result of an antagonistic mode of action by saralasin. This is in contrast to the present study, where an agonistic mode of action by saralasin resulted in an increase in vascular resistance. An actual increase

in arterial pressure with saralasin might therefore have occurred in these animals, had not cardiovascular reflexes initiated a compensatory fall in cardiac output.

The report of Stubbs, et al and the present study, indicate that the renin-angiotensin system may mediate oestrogen-induced disturbances in cardiovascular function by more than one pathway. The difference in findings between these two studies may well be due to differences in the nature of the oestrogen preparation used. Stubbs, et al used the synthetic oestrogen - enovid, whereas the natural oestrogen - oestrone has been used in the present study. Moreover, the studies which have reported increases in angiotensin II receptor number with oestrogen treatment (47, A.Messenger:pers.comm.)also used natural oestrogens.

Under normal circumstances, angiotensin II exerts an inhibitory effect on renin secretion, through a direct negative feedback mechanism. This effect is believed to be mediated by a vascular receptor in the afferent renal arteriole, or by a direct effect on the juxtaglomerular cells. In the normal, anaesthetised rat, saralasin causes an increase in renin secretion (164). This effect has been interpretated to represent blockade of the negative feedback pathway described above. In the presence of an increase in renin substrate synthesis, this effect of saralasin would explain the dramatic increases in both plasma renin activity and concentration noted in oestrogen treated rats after saralasin infusion.

In conclusion, saralasin has been found to exhibit intrinsic activity in the oestrogen treated rat, which has a marked expression on the renal vasculature. This effect did not permit any conclusions as to the contribution of angiotensin II

to haemodynamic function in the oestrogen treated rat. It has been suggested that the intrinsic activity of saralasin was the result of an oestrogen-induced increase in vascular angiotensin II receptor number. This effect of oestrogen may contribute to an increase in renal vascular resistance in the oestrogen treated rat.

Chapter 6

A COMPARISON OF THE CIRCULATORY AND HORMONAL RESPONSES TO OESTROGEN TREATMENT BETWEEN THE SPONTANEOUSLY HYPERTENSIVE RAT AND ITS NORMOTENSIVE COUNTERPART

6.1 INTRODUCTION

Disturbances in haemodynamic homeostasis - manifested as hypertension - are a recognised complication in women ingesting oestrogen-containing oral contraceptives (33, 34, 165, 166). These compounds have also been shown to exacerbate pre-existing hypertension (165, 166). As a consequence of this latter observation, ethical considerations have precluded detailed studies of the effect of oestrogen on cardiovascular function in hypertensive humans.

Adequate animal models for human diseases form important elements in the experimental study of their pathogenesis. Animal models for human diseases that are caused exogenously, such as infectious diseases and nutritional deficiency, have been relatively easy to establish. Hereditary hypertension, on the other hand, is multifactorial in nature, reflecting an interaction between genes and the environment. Thus, development of a satisfactory animal model of human hypertension represents a particularly difficult undertaking. The development of strains of hypertensive rats in the late 1950's and early 1960's (167, 168) heralded an important breakthrough in hypertension research. These strains have gradually replaced experimentally induced hypertensive animal models such as renal and deoxycorticosterone acetate (DOCA) hypertension, which had been preponderantly used for hypertension research.

Reports of the effects of oestrogen on cardiovascular function in animal models of hypertension are few in number, and inconsistent in their findings. Where hypertension has been experimentally induced, oestrogens have been reported either to exacerbate (169), or to have no effect (170) on the

hypertension. Similarly, in genetically hypertensive rats, oestrogen has been shown either to enhance hypertension when given alone (171) or in conjunction with a high salt diet (170), or to have no effect on blood pressure (170). These animal studies, being confined to blood pressure measurements alone, do not provide a complete haemodynamic picture - preventing conclusions as to the underlying mechanisms responsible for the blood pressure changes.

The objectives of the present study were two-fold. Firstly, to compare the influence of oestrogen on haemodynamic function and plasma and renal renin in the spontaneously hypertensive rat (SHR) and their normotensive Wister Kyoto (WKY) counterparts, and secondly, to investigate specifically the role of angiotensin II in any observed changes - by the use of the angiotensin converting enzyme inhibitor, captopril.

6.2 METHODS

Adult female spontaneously hypertensive rats (SHR: 190 - 235 g) and their normotensive Wistar Kyoto counterparts (WKY: 180 - 245 g) were used throughout the study (Olac Ltd, England). Animals were housed and fed as described in Chapter 3.

Oestrone acetate in ethyl oleate (4mg per ml) was used to give subcutaneous injections of 1 mg oestrogen per day for 10 days, to both SHR and WKY rats. Control animals received an injection of vehicle alone.

24-hours after receiving their last injection of vehicle, vehicle-injected rats were prepared for experimental analysis. After receiving their last injection of oestrogen, oestrogen treated rats were split into two batches. The first batch of

animals was prepared for experimental analysis as for vehicle-injected animals. The second batch of rats were fasted overnight, prior to receiving a single dose (P.O.) of either Captopril (30 mg per kg) or 0.9% saline vehicle (1 ml) by gavage. Rats were then prepared for experimental analysis. The dose of captopril used here has been shown to inhibit the pressor response to 100 ng per kg of angiotensin II (i.v.) within 10 minutes, an effect which lasts for 2-3 hours (172).

Haemodynamic and renin-angiotensin variables and the packed cell volume of blood were measured as described in chapter 2. For the second batch of rats, haemodynamic measurements were made approximately 90 minutes after administration of captopril or saline.

6.3 RESULTS

The injection of vehicle alone did not alter any of the parameters under study in both SHR and WKY rats (except for the packed cell volume (Table 6.1)). For statistical purposes therefore, data from unmanipulated rats has been combined with data from the corresponding strain of vehicle-injected rats. The two groups of animals thus formed are referred to as non-oestrogen treated controls.

Comparison of the Data Obtained From the Non Oestrogen Treated Control Groups of WKY and SHR (Table 6.2)

Mean arterial pressure and peripheral and renal vascular resistance were significantly greater in the SHR compared to WKY rats. There was no difference in cardiac output, stroke volume, renal blood flow and packed cell volume between the two strains of rats.

Renal renin concentration was significantly lower in the SHR. There was no other significant differences in the remaining parameters of the renin-angiotensin system under study.

The above observations are in agreement with published data (173).

The Effects of Oestrogen in WKY (Table 6.3)

Haemodynamics

Mean arterial pressure rose from a control value of 120 ± 6 to 140 ± 8 mm Hg ($p < 0.001$) after oestrogen treatment. Cardiac output and stroke volume increased from 300 ± 46 and 0.77 ± 0.10 to 369 ± 39 ml kg^{-1} min^{-1} ($p < 0.05$) and 0.95 ± 0.10 ml $beat^{-1}$ kg^{-1} ($p < 0.05$) respectively. Oestrogen treatment had no effect on total peripheral resistance (33,660 ± 5,141 v 31,051 ± 3,332 dyn cm^{-5} sec^{-1} kg^{-1}).

A fall in renal blood flow - from 7.71 ± 1.00 to 6.25 ± 0.57 ml g^{-1} min^{-1} ($p < 0.05$) - and a rise in renal vascular resistance - from 1,282 ± 131 to 1,830 ± 204 dyn cm^{-5} sec^{-1} kg^{-1} ($p < 0.001$) - took place as a result of oestrogen treatment.

Renin-Angiotensin System

Plasma renin activity increased from a control value of 2.29 ± 0.43 to 4.73 ± 2.10 ng Ang I ml^{-1} hr^{-1} ($p < 0.05$) after oestrogen treatment. Whilst oestrogen treatment had no effect on plasma renin concentration (9.13 ± 2.12 v 8.39 ± 2.9 ng Ang I ml^{-1} hr^{-1}), renal renin concentration fell from 6,248 ± 800 to 4,616 ± 907 ng Ang I mg^{-1} hr^{-1} ($p < 0.05$) with oestrogen.

Packed Cell Volume

Oestrogen treatment reduced the packed cell volume from a control value of 32.2 ± 0.6 to 27.4 ± 1.1% ($p < 0.001$)

The Effects of Oestrogen in SHR (Table 6.4)

Haemodynamics (Figure 6.1)

The administration of oestrogen resulted in a fall in mean arterial pressure, from a control value of 175 ± 5 to 164 ±

6 mm Hg ($p < 0.05$). Cardiac output and stroke volume increased from 248 ± 51 and 0.67 ± 0.13 to 369 ±90 ml min^{-1} kg^{-1} ($p < 0.05$) and 1.21 ± 0.33 ml $beat^{-1}$ kg^{-1} ($p < 0.01$) respectively. Total peripheral resistance decreased from 67,611 ± 16,168 to 41,650 ± 10,856 dyn cm^{-5} sec^{-1} kg^{-1} ($p < 0.05$) after oestrogen.

A fall in renal blood flow - from 7.23 ± 1.12 to 5.22 ± 0.55 ml min^{-1} g^{-1} ($p < 0.05$) - and a rise in renal vascular resistance - from 2,078 ± 270 to 2,584 ± 294 dyn cm^{-5} sec^{-1} kg^{-1}($p < 0.05$) - occurred as a result of oestrogen treatment.

Renin-Angiotensin System (Figure6 .2)

Oestrogen treatment increased plasma renin activity from a control value of 3.34 ± 1.02 to 6.20 ± 1.39 ng Ang I ml^{-1} hr^{-1} ($p < 0.01$). Both plasma and renal renin concentration were reduced after oestrogen treatment, from 10.21 ± 3.29 to 7.47 ± 1.78 ng Ang I ml^{-1} hr^{-1} and 3,182 ± 329 to 2,941 ± 261 ng Ang I mg^{-1} hr^{-1} respectively. These reductions did not, however, achieve statistical significance.

Packed Cell Volume (Figure6 .2)

A fall in the packed cell volume from 29.9 ± 0.5 to 26.9 ± 1.1% ($p < 0.001$) took place as a result of oestrogen treatment.

The haemodynamic, renin-angiotensin and packed cell volume responses of WKY and SHR to oestrogen are summarised in Table 6.5

The Effects of Captopril on Haemodynamics and the Packed Cell Volume of Oestrogen Treated WKY. (Table6 .6 and Figure 6.3)

Systemic Haemodynamics

Captopril reduced the mean arterial pressure of oestrogen treated WKY from a median value of 151, with a range of 150-167 (saline vehicle controls), to 131 ± 9 mm Hg ($p < 0.01$). This latter value was greater than the 120 ± 6 mm Hg value noted

for non-oestrogen treated control WKY ($F = 4.87$, $p < 0.05$).

Although captopril decreased total peripheral resistance from 41,574 ± 8,361 to 31,131 ± 6,933 dyn cm^{-5} sec^{-1} kg^{-1}, this change did not achieve statistical significance. Cardiac output was not affected by the administration of captopril (315 ± 59 v. 399 ± 169 ml min^{-1} kg^{-1}).

Renal Haemodynamics

Captopril caused an increase in renal blood flow and a decrease in renal vascular resistance, from 5.98 ± 0.94 and 2,157 ± 376 to 8.24 ± 0.80 ml min^{-1} g^{-1} ($p < 0.01$) and 1,299 ± 149 dyn cm^{-5} sec^{-1} kg^{-1} ($p < 0.001$) respectively. These latter values were similar to those noted in control (non-oestrogen treated) animals (7.71 ± 1.00 ml min^{-1} g^{-1} and 1,282 ± 131 dyn cm^{-5} sec^{-1} kg^{-1}).

Packed Cell Volume

The packed cell volume remained unchanged after administration of captopril (28.9 ± 1.6 v. 28.8 ± 1.4%).

The Effects of Captopril on Haemodynamics and the Packed Cell Volume of Oestrogen Treated SHR (Table 6.7 and Figure 6.4)

Systemic Haemodynamics

Captopril had no effect on arterial pressure (154 ± 14 v. 141 ± 22 mm Hg), cardiac output (363 ± 100 v. 319 ± 31 ml min^{-1} kg^{-1}) or total peripheral resistance (35,840 ± 7,597 v. 36,270 ± 5,439 dyn cm^{-5} sec^{-1} kg^{-1}).

Renal Haemodynamics

The administration of captopril resulted in an increase in renal blood flow - from 6.24 ± 0.86 to 10.63 ± 0.61 ml min^{-1} g^{-1} ($p < 0.001$) - and a decrease in renal vascular resistance - from 2,030 ± 435 to 1,084 ± 165 dyn cm^{-5} sec^{-1} kg^{-1} ($p < 0.001$).

Thus captopril caused a rise in renal blood flow in

oestrogen treated SHR to a level above that noted in non-oestrogen treated SHR ($F = 11.69$, $p < 0.01$), and a fall in renal vascular resistance to a level below that of non-oestrogen treated SHR ($F = 17.15$, $p < 0.001$).

Packed Cell volume

The packed cell volume was not affected by captopril (27.6 ± 1.0 v. 28.0 ± 0.6%).

6.4 DISCUSSION

The findings from this study have demonstrated that whereas oestrogen caused a rise in arterial pressure in WKY, it induced a fall in pressure in the SHR. A reduction in renal blood flow and an increase in renal vascular resistance were noted in both WKY and SHR after oestrogen. These haemodynamic changes were accompanied by a reduction in the packed cell volume and an increase in plasma renin activity, in both strains of rats. Administration of captopril resulted in a fall in mean arterial pressure in the oestrogen treated WKY, but was without effect in the SHR. In the oestrogen treated WKY, captopril returned renal blood flow to the pretreatment level. Captopril also increased renal blood flow in the oestrogen treated SHR, but to a level which was still significantly greater than that observed in non-oestrogen treated SHR.

The haemodynamic, packed cell volume and plasma renin activity and renal renin concentration responses of WKY to oestrogen match the responses of the Sprague-Dawley rats (given the same amount of oestrogen) described in chapter 3. Although a reduction in plasma renin concentration was also noted in WKY after oestrogen, this change was not statistically significant. This finding does not agree with the marked reduction in plasma renin concentration noted in the Sprague-Dawley animals referred

TABLE 6.1. Effects of Vehicle (ethyl oleate) Injection on Haemodynamics, the Renin-Angiotensin System and the Packed Cell Volume, in The Spontaneously Hypertensive Rat.

Data are presented as a mean ± 95% C.L. Data were analysed using a one way ANOVA. The probability value of the test statistic - F is indicated in the test column.

PARAMETER	NON TREATED		VEHICLE - 0.25 ml per day for 10 days		TEST
Mean Arterial Pressure mm Hg	180±6	n=6	172±5	n=10	F=3.83 NS
Heart Rate Beats per min	371±29	n=6	367±15	n=10	F=0.08 NS
Cardiac Output ml min^{-1} kg^{-1}	291±57	n=6	219±72	n= 9	F=1.94 NS
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.78±0.13	n=6	0.60±0.2	n= 9	F=1.80 NS
Total Peripheral Resistance dyn cm^{-5} $sec^{-1}kg^{-1}$	52,620±12,142	n=6	77,605±24,106	n= 9	F=2.43 NS
Renal Blood Flow ml min^{-1} g^{-1}	6.84±0.82	n=6	7.52±1.90	n= 8	F=0.32 NS
Renal Vascular Resistance dyn cm^{-5} $sec^{-1}kg^{-1}$	2,141±216	n=6	2,031±459	n= 8	F=0.14 NS
Plasma Renin Activity ng Ang I $ml^{-1}hr^{-1}$	2.31±0.67	n=6	4.03±1.51	n= 9	F=3.00 NS
Plasma Renin Concentration ng Ang I $ml^{-1}hr^{-1}$	7.29±2.34	n=6	12.15±4.98	n=9	F=2.17 NS
Renal Renin Concentration ng Ang I $mg^{-1}hr^{-1}$	2,802±186	n=6	3,410±465	n= 9	F=3.64 NS
Packed Cell Volume %	33.4±0.7	n=6	29.9±0.5	n=9	F=61.16 ***

TABLE 6.2. Haemodynamic, Renin-Angiotensin and Packed Cell Volume Data from Non Oestrogen Treated Normotensive Wistar and Spontaneously Hypertensive Rats.
Legend as described in Table 6.1

PARAMETER	NORMOTENSIVE (n = 10)	SPONTANEOUSLY HYPERTENSIVE (n = 15)	TEST
Mean Arterial Pressure mm Hg	120±6	175±5	F = 218 •••
Heart Rate beats per min	387±15	368±14	F = 3.05 NS
Cardiac Output ml min^{-1} kg^{-1}	300±46	248±51	F = 1.98 NS
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.77±0.10	0.67±0.13	F = 1.08 NS
Total Peripheral Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	33,660±5,141	67,611±16,168	F = 10.67 ••
Renal Blood Flow ml min^{-1} g^{-1}	7.71±1.00	7.23±1.12	F = 0.36 NS
Renal Vascular Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	1,282±131	2,078±270	F = 21.0 •••
Plasma Renin Activity ng Ang I ml^{-1} hr^{-1}	2.29±0.43	3.34±1.02	F = 1.37 NS
Plasma Renin Concentration ng Ang I ml^{-1} hr^{-1}	9.13±2.12	10.21±3.29	F = 0.29 NS
Renal Renin Concentration ng Ang I $mg^{-1}hr^{-1}$	6,248±800	3,182±329	F = 68.48 •••
Packed Cell Volume %	32.2±0.63	31.3±1.1	F = 1.69 NS

TABLE 6.3. The Effects of 10mg Oestrone Acetate on Haemodynamics, the Renin-Angiotensin System and Packed Cell Volume in Normotensive Wister Rats. Legend as described in Table 6.1

PARAMETER	CONTROL (n = 10)	OESTROGEN TREATMENT (n = 10)	TEST
Mean Arterial Pressure mm Hg	120±6	140±8	F = 16.22 •••
Heart Rate beats per min	387±15	389±20	F = 0.04 NS
Cardiac Output ml min^{-1} kg^{-1}	300±46	369±39	F = 5.00 •
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.77±0.10	0.95±0.10	F = 5.70 •
Total Peripheral Resistance dyn cm^{-5} sec^{-1} kg^{-1}	33,660±5,141	31,051±3,332	F = 0.07 NS
Renal Blood Flow ml g^{-1} min^{-1}	7.71±1.00	6.25±0.57	F = 6.28 •
Renal Vascular Resistance dyn cm^{-5} $sec^{-1}kg^{-1}$	1,282±131	1,830±204	F = 19.72 •••
Plasma Renin Activity ng Ang I ml^{-1} hr^{-1}	2.29±0.43	4.73±2.10	F = 5.54 •
Plasma Renin Concentration ng Ang I ml^{-1} hr^{-1}	9.13±2.12	8.39±2.90	F = 0.17 NS
Renal Renin Concentration ng Ang I $mg^{-1}hr^{-1}$	6,248±800	4,616±907	F = 7.00 •
Packed Cell Volume %	32.2±0.6	27.4±1.1	F = 55.68 •••

TABLE 6.4. The Effects of 10 mg Oestrone Acetate on Haemodynamics, the Renin-Angiotensin System and Packed Cell Volume, in the Spontaneously Hypertensive Rat.

Legend as described in Table 6.1 ◆- Data represents vehicle injected animals alone.

PARAMETER	CONTROL		OESTROGEN TREATED n = 10	TEST
Mean Arterial Pressure mm Hg	175±5	n=16	164±6	F= 7.69 •
Heart Rate beats per min	368±14	n=16	311±18	F=24.92 •••
Cardiac Output ml min^{-1} kg^{-1}	248±51	n=15	369±90	F= 6.02 •
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.67±0.13	n=15	1.21±0.33	F=11.04 ••
Total Peripheral Resistance dyn cm^{-5} sec^{-1} kg^{-1}	67,611±16,168	n=15	41,650±10,856	F= 5.45 •
Renal Blood Flow ml min^{-1} g^{-1}	7.23±1.12	n=14	5.22±0.55	F= 7.67 •
Renal Vascular Resistance dyn cm^{-5} sec^{-1} kg^{-1}	2,078±270	n=14	2,584±294	F=5.97 •
Plasma Renin Activity ng Ang I ml^{-1} hr^{-1}	3.34±1.02	n=15	6.20±1.39	F=10.99 ••
Plasma Renin Concentration ng Ang I ml^{-1} hr^{-1}	10.21±3.29	n=15	7.47±1.78	F= 1.54 NS
Renal Renin Concentration ng Ang I mg^{-1} hr-1	3,182±329	n=16	2,941±261	F= 1.03 NS
Packed Cell Volume %	◆ 29.9±0.5	n= 9	26.9±1.1	F=22.9 •••

TABLE 6.5. The Effects of Oestrone Acetate on Haemodynamics, the Renin-Angiotensin System and the Packed Cell Volume, in Normotensive Wistar and Spontaneously Hypertensive Rats. Direction of arrow indicates direction of change as compared to control: an inclined arrow indicates a slight change, a horizontal arrow no difference.

PARAMETER	NORMOTENSIVE	SPONTANEOUSLY HYPERTENSIVE
Mean Arterial Pressure	↑	↓
Heart Rate	→	↓
Cardiac Output	↑	↑
Stroke Volume	↑	↑
Total Peripheral Resistance	→	↓
Renal Blood Flow	↓	↓
Renal Vascular Resistance	↑	↑
Plasma Renin Activity	↑	↑
Plasma Renin Concentration	↘	↘
Renal Renin Concentration	↓	↘
Packed Cell Volume	↓	↓

TABLE 6.6 The Effects of a Single P.O. Dose of Captopril (30mg per kg) on Haemodynamics and the Packed Cell Volume of Oestrogen Treated Normotensive Rats. Legend as described in Table 6.1

PARAMETER	SALINE DOSED CONTROLS (n = 7)	CAPTOPRIL DOSED (n = 8)	TEST
Mean Arterial Pressure mm Hg	151; 150-167	131 ± 9	Z = 3.07 ••
Heart Rate beats per min	395 ± 22	408 ± 22	F = 0.67 NS
Cardiac Output ml min^{-1} kg^{-1}	315 ± 59	399 ± 169	F = 0.76 NS
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.79 ± 0.13	0.97 ± 0.37	F = 0.65 NS
Total Peripheral Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	41,574 ± 8,361	31,131 ± 6,933	F = 3.61 NS
Renal Blood Flow ml min^{-1} g^{-1}	5.98 ± 0.94	8.24 ± 0.80	F = 13.26 ••
Renal Vascular Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	2,157 ± 375	1,299 ± 149	F = 19.00 •••
Packed Cell Volume %	28.9 ± 1.6	28.8 ± 1.4	F = 0 NS

TABLE 6.7 The Effects of a single P.O. Dose of Captopril (20mg per kg) on Haemodynamics and the Packed Cell Volume of Centrogen Treated Spontaneously Hypertensive Rats. Legend as described in Table 6.1

PARAMETER	SALINE DOSED CONTROLS (n = 5)	CAPTOPRIL DOSED (n = 5)	TEST
Mean Arterial Pressure mm Hg	154 ± 14	141 ± 22	F = 1.02 NS
Heart Rate beats per min.	331 ± 35	355 ± 27	F = 1.06 NS
Cardiac Output ml min^{-1} kg^{-1}	363 ± 100	319 ± 31	F = 0.68 NS
Stroke Volume ml $beat^{-1}$ kg^{-1}	1.09 ± 0.24	0.90 ± 0.12	F = 1.96 NS
Total Peripheral Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	35,840 ± 7,595	36,270 ± 8,439	F = 0 NS
Renal Blood Flow ml min^{-1} g^{-1}	6.24 ± 0.86	10.63 ± 0.61	F = 65.69 •••
Renal Vascular Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	20,030 ± 435	1,084 ± 165	F = 15.94 ••
Packed Cell Volume %	27.6 ± 1.0	28.0 ± 0.6	F = 0.56 NS

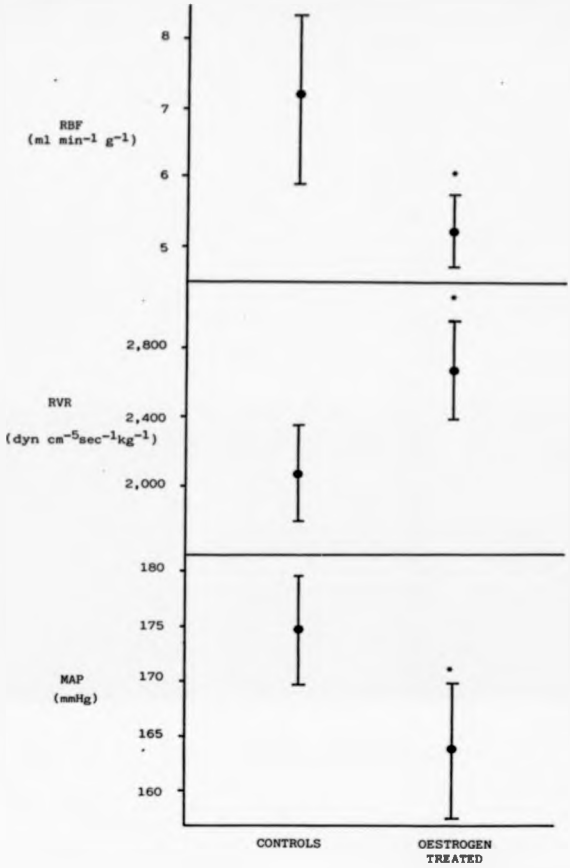


Figure 6.1 The Effects of 10mg of Oestrone Acetate on the Renal Blood Flow (RBF), Renal Vascular Resistance (RVR) and Mean Arterial Pressure (MAP) of Spontaneously Hypertensive Rats

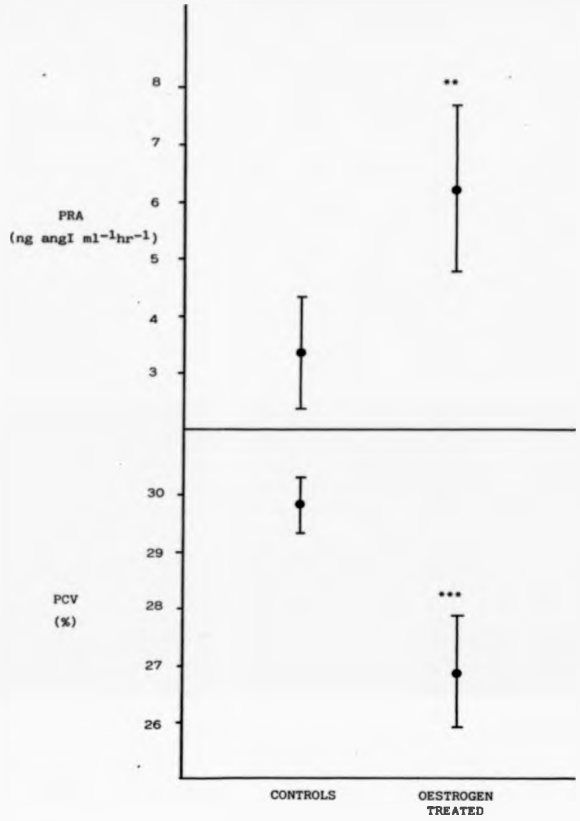


Figure 6.2 The Effects of 10mg of Oestrone Acetate on the Plasma Renin Activity (PRA) and Packed Cell Volume (PCV) of Spontaneously Hypertensive Rats

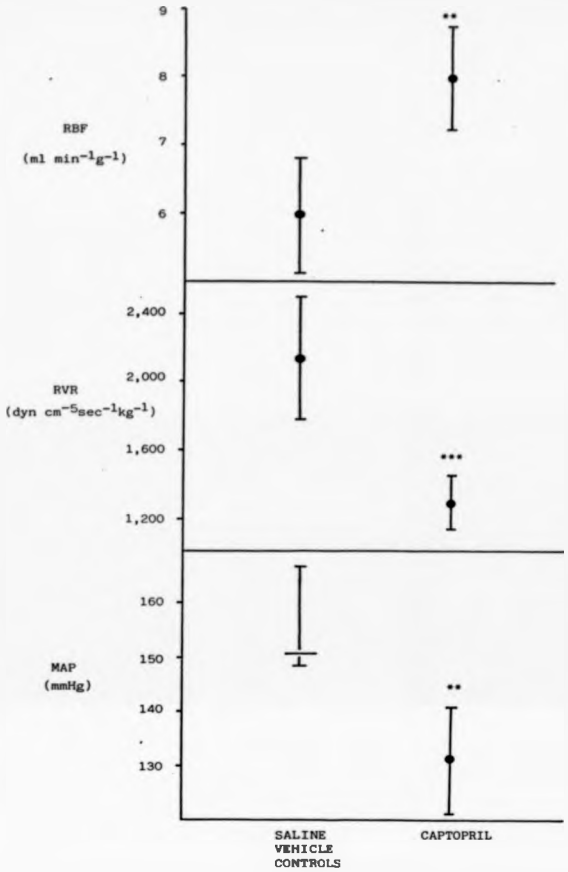


Figure 6.3 The Effects of Captopril (P.O. -30mg per Kg) on the Renal Blood Flow, Renal Vascular Resistance and Mean Arterial Pressure of Normotensive Wistar Rats which had Received 10mg of Oestrone Acetate

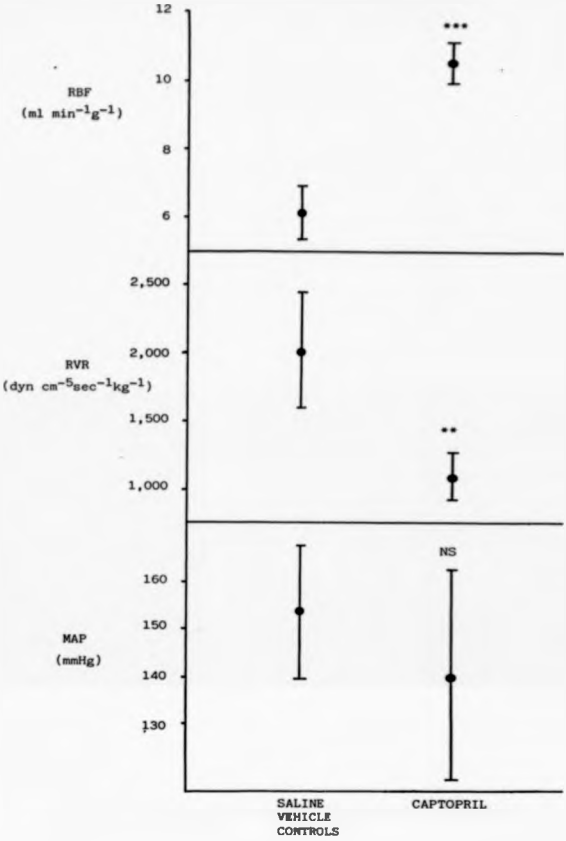


Figure 6.4 The Effects of Captopril (P.O. - 30mg per kg) on the Renal Blood Flow, Renal Vascular Resistance and Mean Arterial Pressure of Spontaneously Hypertensive Rats which had Received 10mg of Oestrone Acetate

to above. This observation may be accounted for by the fact that the plasma renin concentrations noted in unmanipulated Sprague-Dawley rats were far greater than the values recorded for unmanipulated WKY. Since the radioimmunoassay used to measure this variable was developed using Sprague-Dawley rats, it may not have been sensitive enough to detect a decrease in renin concentration in WKY. On the other hand, failure to suppress renin secretion in the presence of an increase in circulating angiotensin II has been considered by Saruta, et al (174) to be a causal factor leading to the establishment of oestrogen-associated hypertension. An impairment of the negative feedback relationship between circulating angiotensin II and renin secretion may therefore contribute to the development of the increased arterial pressure noted in WKY after oestrogen treatment. Consistent with this hypothesis is the observation that WKY rats exhibited a larger mean rise in arterial pressure in response to oestrogen treatment than did Sprague-Dawley rats (+ 20 v. + 16 mm Hg).

The physiopathological importance of changes in the renin-angiotensin system to alterations in haemodynamics in oestrogen treated normotensive Sprague-Dawley and WKY rats appear to be essentially similar. The findings from chapter 3 suggested that an oestrogen-induced stimulation of the renin-angiotensin system was involved in establishing aberrations in both systemic and renal haemodynamics, although no definate conclusions concerning causation could be drawn from these findings. In the present study, captopril returned the renal blood flow of rats with an oestrogen-induced reduction in renal blood flow to the pretreatment level, through a decrease in renal vascular resistance. This observation provides good evidence to support the hypothesis that an increase in the concentration of

angiotensin II in the intrarenal circulation was the principal event leading to the establishment of a reduced renal perfusion in the oestrogen treated normotensive rat. The reduction in arterial pressure noted in WKY rats after captopril administration clearly demonstrates the involvement of angiotensin II in the maintenance of the hypertension noted in these animals. The fact that the arterial pressure of these captopril treated rats was still significantly larger than the pressure noted in non-oestrogen treated animals reinforces the concept outlined previously, that oestrogen-associated hypertension is multifactorial in nature - the other major factor to be identified by this investigation being an expanded plasma volume.

The SHR exhibited a fundamentally different blood pressure response to oestrogen treatment compared to its normotensive counterpart. In the SHR, blood pressure did not become elevated, but actually fell after oestrogen treatment. This finding does not agree with previous reports, which have shown a rise in blood pressure in genetically hypertensive rats with oestrogen (171,177). However, a single author, using the New Zealand strain of genetically hypertensive rat, has reported this finding. In the only published report using SHR, Hoeg, et al (178) have reported that chronic administration of oestrogen to young SHR, for a period of 8 weeks, reduced the level of hypertension attained. Whilst this report is consistent with the present finding, it is unlikely that oestrogen attenuated the development of hypertension in the SHR used in this study. The hypertension of these animals, at approximately 20 weeks of age, is well established, will increase only marginally with advancing age, and not at all over a period of 10 days (168).

The effect of oestrogen on the blood pressure of SHR therefore almost certainly reflects an effect of the steroid on established hypertension.

The reduction in mean arterial pressure noted in SHR after oestrogen treatment took place in spite of an increase in cardiac output. As for WKY, this increase was presumably due to an expanded plasma volume - consequent to oestrogen-mediated salt and water retention (175,176). The fall in arterial pressure must therefore have been the result of the observed reduction in peripheral vascular resistance. This reduction occurred in spite of an increase in plasma renin activity - which might have been expected to promote an increase in vascular resistance. This situation is in contrast to that encountered in WKY, where peripheral vascular resistance remained unchanged in the presence of an increased cardiac output. Here, elevated plasma renin activity probably prevented a compensatory fall in peripheral vascular resistance. Taken together, these findings suggest that the different blood pressure response to oestrogen treatment, between SHR and WKY, may reflect in part a decline in the responsiveness of peripheral resistance vessels to angiotensin II in the spontaneously hypertensive animal. Support for this hypothesis may be derived from the reported observation that the SHR exhibits a reduced vasoconstrictor response when subjected to acute haemorrhage (182). Similarly, hypertensive humans have been reported to have abnormalities in responsiveness of the renin-angiotensin system (179,180), including a blunted vascular response to renin (181).

The hypothesis outlined above, may well account for a reduction in peripheral vascular resistance in the presence of

an increased plasma renin activity. It cannot, however, explain the fact that the reduction in peripheral vascular resistance noted in oestrogen treated SHR was to such a degree that it resulted in a fall in arterial pressure. Two possible mechanisms may explain this observation. Firstly, the administration of oestrogen to SHR might have induced a marked increase in systemic vasodilator substances. In this context, oestrogen has been shown to enhance the vascular prostaglandin synthesis, either through a direct stimulatory effect (44-46), or through its effect on the renin-angiotensin axis (185). Further, genetically hypertensive rats exhibit an enhanced vasodepressor response to intravenous infusion of both prostaglandin precursors (183) and prostaglandins (184) compared to their normotensive counterparts. Moreover, experiments undertaken in this laboratory have shown that chronic inhibition of prostaglandin synthesis can prevent a fall in arterial pressure in the oestrogen treated SHR (C.Stonier - personal communication). Secondly, although there are no data from the SHR, an increase in peripheral venous distensibility, resulting in a loss in venous tone, has been reported in women ingesting oestrogen-containing oral contraceptives (186).

Collectively, the data described above, indicate that the reduction in arterial pressure noted in the SHR after oestrogen may have been the result of prostaglandin-mediated vasodilation in the presence of peripheral arteriolar hyporeactivity to angiotensin II, and/or a direct effect of oestrogen on peripheral arterioles, resulting in a loss in vascular tone.

In contradistinction to the marked differences in peripheral haemodynamic responses between oestrogen.treated WKY and SHR, the intrarenal response of the two strains of rat was

similar, both showing a reduction in renal blood flow with oestrogen. Likewise, these reductions in renal perfusion were both due to an increased plasma renin activity, which led to an increase in renal vascular resistance. This proposition is supported by the observation that administration of captopril to oestrogen treated WKY and SHR resulted in a marked reduction in renal vascular resistance in both strains, thereby increasing renal blood flow. As for WKY therefore, an increased concentration of angiotensin II in the intrarenal circulation appears to be the causal factor responsible for a reduction in renal blood flow in the SHR after oestrogen treatment.

It was of interest to note that in the oestrogen treated SHR, unlike WKY, captopril increased renal blood flow to a level which was significantly greater than that noted in non-oestrogen treated rats. This effect may have occurred as a result of the bradykinin potentiating property of captopril (152), and is consistent with the reported increase in sensitivity to the vasodepressor effect of the antihypertensive prostaglandin endocrine system, seen in the SHR compared to its normotensive counterpart (183, 184).

From the oestrogen-induced changes in haemodynamics described above, it is clear that oestrogen has exerted opposite effects on peripheral and renal resistance vessels in the SHR. A fall in peripheral vascular resistance may have resulted from a stimulatory effect of oestrogen on prostaglandin-mediated vasodilation of peripheral resistance vessels, in the presence of a reduced vascular responsiveness to angiotensin II. One might anticipate that this effect would also lead to a fall in renal vascular resistance. Clearly this was not the case. The renal vasculature is, however, especially sensitive to the

vasoconstrictor effect of angiotensin II (6). Moreover, whilst prostaglandins have been found to exhibit potent vasodilatory properties in several species, including man, anomalous responses have been observed in the rat. In this species, whereas prostaglandins vasodilate peripheral resistance vessels, they appear to vasoconstrict the renal vasculature (187). Indeed, McGiff and Quilley (188) feel that this difference constitutes the basis for rejection of the genetically hypertensive rat as a suitable model of human hypertension. In the SHR therefore, a reduction in the responsiveness of peripheral blood vessels to angiotensin II may not extend to the hypersensitive renal vasculature, in which prostaglandins may act as vasoconstrictors. These observations may explain what, at first glance, appear to be divergent vascular resistance changes.

In conclusion, oestrogen has been found to induce a reduction in renal blood flow in both WKY and SHR. This reduction appeared to be the result of angiotensin II - mediated constriction of renal resistance vessels in both strains of rats. Oestrogen exerted prohypertensive and antihypertensive effects in WKY and SHR respectively. This differing circulatory response was due, in part, to angiotensin II - mediated vasoconstriction in WKY, and a reduction in peripheral vascular resistance in SHR. It has been suggested that this latter effect was the result of prostaglandin-mediated vasodilation of peripheral resistance vessels in the presence of a reduced vascular responsiveness to angiotensin II.

Chapter 7

THE EFFECTS OF PROSTAGLANDIN SYNTHESIS INHIBITION ON RENAL AND SYSTEMIC HAEMODYNAMICS IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS AFTER OESTROGEN TREATMENT

7.1 INTRODUCTION

The contribution of prostaglandins to the modulation of renal haemodynamics and blood pressure control under both normal circumstances (18, 19, 31) and a number of physiopathological states, such as sodium deprivation (27) and hypertension (32), has been described during the course of this investigation. In the SHR, an increased elaboration of vasodilatory prostaglandins may be evoked in response to the hypertensive state. Thus under normal circumstances, inhibition of prostaglandin synthesis has no effect on arterial pressure in WKY, whereas it results in an increase in arterial pressure in SHR (192). The findings from the preceding chapter suggest that prostaglandin synthesis may also be a major determinant of blood pressure in the SHR during oestrogen treatment.

Pharmacological interruption of prostaglandin synthesis by aspirin-like drugs, such as indomethacin and meclofenamate, is the standard technique used to investigate the physiological role of prostaglandins. These substances inhibit the action of tissue cyclo-oxygenase (see p.7), thereby preventing the formation of prostaglandins (189).

The objective of this study therefore, was to investigate the contribution of prostaglandins to cardiovascular function in the oestrogen treated SHR and WKY rat, by use of the prostaglandin synthesis inhibitor, indomethacin.

7.2 METHODS

Experimental Procedure

Oestrone acetate in ethyl oleate (4 mg per ml) was used to give subcutaneous injections of 1 mg of oestrogen per day for 10 days to both SHR (160-200 g) and WKY (188-279 g).

24-hours after receiving their last injection of oestrogen, rats were prepared for experimental analysis as described in chapter 2. Rats were infused intra-arterially via the femoral catheter, with either indomethacin (total dose- 6 mg per kg of body weight) or vehicle (total volume- 0.340 ml) over a 10 minute period. Approximately 45 minutes after initiation of infusion of either indomethacin or vehicle, haemodynamic variables and the packed cell volume were measured as described in chapter 2.

Preparation of Indomethacin

Indomethacin has a low solubility in water, so that preparation of the drug by dissolving it in an alkali is necessary where the intravenous route of administration is to be used. However, indomethacin is unstable in alkaline solutions, and special attention must therefore be given to preparation of the drug (190).

Indomethacin (Sigma) was dissolved in 0.05 M sodium carbonate at room temperature, and the pH rapidly adjusted to approximately 7.5 with 1 M hydrochloric acid. The solution was immediately split into the required number of 3 ml aliquots, frozen, and stored in the dark at -20° C for no longer than 20 days. A solution of sodium carbonate (0.05 M, pH 7.5 and stored at -20° C) acted as vehicle. Aliquots of the drug or vehicle were warmed to 37° C immediately prior to infusion.

It has been shown that indomethacin preparations of the type described above are stable at -20° C for at least 100 days, and at

room temperature for at least 1 hour (190).

The dose of indomethacin selected for this study (6 mg per kg) has been shown to cause significant inhibition of renal prostaglandin synthesis in the rat (>90% reduction in urinary PG excretion (191)).

7.3 RESULTS

Effects of Indomethacin in Oestrogen Treated [illegible] (Table 7.1 and Figure 7.1)

Systemic Haemodynamics

Administration of indomethacin had no effect on mean arterial pressure (167 ± 9 v. 158 ± 7 mm Hg), cardiac output (340 ± 59 v. 334 ± 59 ml $min^{-1}kg^{-1}$) or total peripheral resistance (40,728 ± 7,377 v. 39,420 ± 7,215 dyn $cm^{-5}sec^{-1}kg^{-1}$).

Renal Haemodynamics

Renal blood flow increased from 5.35 ± 0.19 to 7.14 ± 1.16 ml $min^{-1}g^{-1}$ ($p < 0.05$), and renal vascular resistance decreased from 2,493 ± 149 to 1,847 ± 386 dyn $cm^{-5}sec^{-1}kg^{-1}$ ($p < 0.05$) after indomethacin.

Packed Cell Volume

The packed cell volume was not influenced by administration of indomethacin (27.3 ± 1.2 v. 27.3 ± 1.6 %).

Effects of Indomethacin in Oestrogen Treated [illegible] (Table 7.2)

Systemic Haemodynamics

Mean arterial pressure (131 ± 7 v. 137 ± 7 mm Hg), cardiac output (309 ± 67 v. 332 ± 33 ml $min^{-1}kg^{-1}$) and total peripheral resistance (36,806 ± 10,892 v. 33,465 ± 4,343 dyn $cm^{-5}sec^{-1}kg^{-1}$) remained unchanged after indomethacin.

Renal Haemodynamics

Renal blood flow increased from 5.63 ± 1.27 to 7.16 ± 0.55 ml $min^{-1}g^{-1}$, and renal vascular resistance decreased from 2,097 ± 802 to 1,539 ± 135 dyn $cm^{-5}sec^{-1}kg^{-1}$ with indomethacin.

However, neither of these changes achieved statistical significance.

Packed Cell Volume

Infusion of indomethacin did not influence the packed cell volume (31.6 ± 1.2 v. 31.6 ± 0.7%).

7.4 DISCUSSION

The findings from this study have demonstrated that in the oestrogen treated rat, inhibition of prostaglandin synthesis has no effect on arterial pressure in either WKY or SHR. This inhibition did, however, result in a significant increase in renal blood flow in SHR. The same direction of change in renal blood flow also occurred in WKY.

The findings from the preceding chapter demonstrated that a reduction in arterial pressure occurred in the SHR after oestrogen treatment. This reduction was due to a marked decrease in peripheral vascular resistance, which took place in spite of an increased plasma renin activity. This observation, coupled with both the known stimulatory effect of oestrogen on vascular prostaglandin synthesis (44-46), and the enhanced sensitivity to prostaglandins shown by SHR compared to WKY (184), suggests that the reduced arterial pressure noted in SHR after oestrogen may have been the result of prostaglandin-mediated vasodilation in the presence of peripheral arteriolar hyposensitivity to the vasoconstrictor action of angiotensin II. The present finding, that administration of indomethacin had no effect on the arterial pressure of oestrogen treated SHR, does not, however, agree with the hypothesis outlined above. This lack of effect of indomethacin may well be accounted for by the acute nature of the dosing regime employed during the current study. Indeed, work done in these laboratories has shown that the simultaneous, chronic administration of oestrogen and indomethacin (subcutaneously) over a period of 10 days, prevented a fall in arterial pressure in SHR (C. Stonier: personal communication).

TABLE 7.1 The Effects of Indomethacin (6 mg per kg of body weight) on Renal and Systemic Haemodynamics in the SHR after Oestrone Acetate (1 mg per day for 10 days) Treatment.

Values are presented as a mean ± 95% confidence limits. Data have been analysed using a one way ANOVA. The probability value of the test statistic- F is indicated in the test column.

PARAMETER	VEHICLE INFUSED CONTROLS (n = 6)	INDOMETHACIN INFUSED (n = 6)	TEST
Mean Arterial Pressure mm Hg	167 ± 9	158 ± 7	F = 2.38 NS
Heart Rate beats per min.	337 ± 17	302 ± 25	F = 5.04 *
Cardiac Output ml $min^{-1}kg^{-1}$	340 ± 59	334 ± 59	F = 0.02 NS
Stroke Volume ml $beat^{-1}kg^{-1}$	1.02 ± 0.24	1.11 ± 0.16	F = 0.41 NS
Total Peripheral Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	40,728 ± 7,377	39,420 ± 7,215	F = 0.06 NS
Renal Blood Flow ml $min^{-1}g^{-1}$	5.35 ± 0.19	7.14 ± 1.16	F = 8.86 *
Renal Vascular Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	2,493 ± 149	1,847 ± 386	F = 9.36 *
Packed Cell Volume %	27.3 ± 1.2	27.3 ± 1.6	F = 0.00 NS

TABLE 7.2 The Effects of Indomethacin (6 mg per kg body weight) on Renal and Systemic Haemodynamics in WKY Rats After Oestrone Acetate (1 mg per day for 10 days) Treatment.

Legend as described in Table 7.1.

PARAMETER	VEHICLE INFUSED CONTROLS (n = 6)	INDOMETHACIN INFUSED (n = 6)	TEST
Mean Arterial Pressure mm Hg	131 ± 7	137 ± 7	F = 1.23 NS
Heart Rate beats per min.	400 ± 14	414 ± 8	F = 3.08 NS
Cardiac Output ml $min^{-1}kg^{-1}$	309 ± 67	332 ± 33	F = 0.35 NS
Stroke Volume ml $beat^{-1}kg^{-1}$	0.78 ± 0.18	0.80 ± 0.08	F = 0.07 NS
Total Preipheral Resistance dyne $cm^{-5}sec^{-1}kg^{-1}$	36,806 ± 10,892	33,465 ± 4,343	F = 0.31 NS
Renal Blood Flow ml $min^{-1}kg^{-1}$	5.63 ± 1.27	7.16 ± 0.55	F = 4.62 NS
Renal Vascular Resistance dyn $cm^{-5}sec^{-1}kg^{-1}$	2,097 ± 802	1,539 ± 135	F = 1.61 NS
Packed Cell Volume %	31.6 ± 1.2	31.6 ± 0.71	F = 0.00 NS

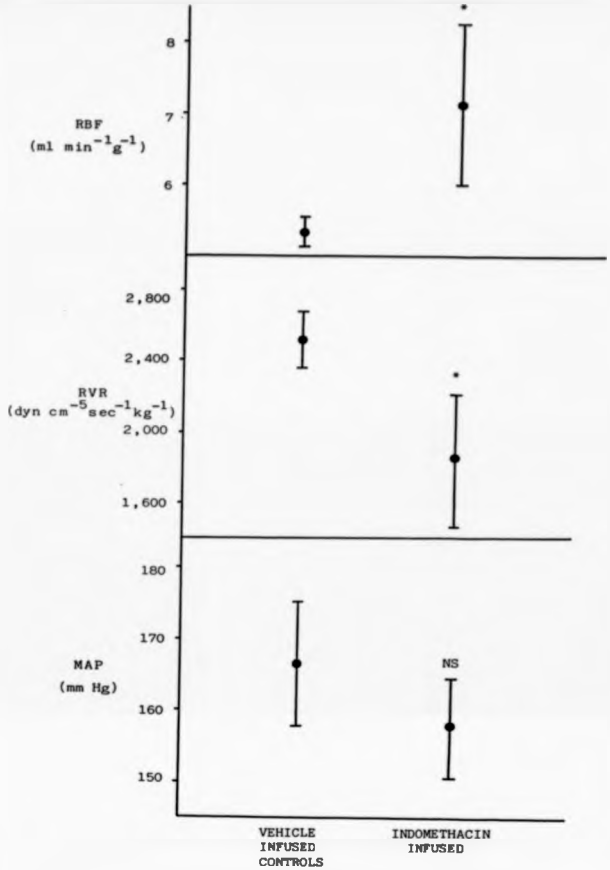


Figure 7.1 The Effects of Indomethacin (6 mg per kg BW) on the Renal Blood Flow (RBF) Renal Vascular Resistance (RVR) and Mean Arterial Pressure (MAP) of Spontaneously Hypertensive Rats Which Had Received 10 mg of Oestrone Acetate

Inhibition of prostaglandin synthesis resulted in an increase in renal blood flow, due to a decreased renal vascular resistance, in oestrogen treated WKY and SHR. This effect is not what one might have anticipated after the elimination of a vasodilatory substance from the renal circulation. It is, however, consistent with the previously described action of prostaglandins such as prostaglandin E_2(187,193), and thromboxane A_2(194), as renal vasoconstrictors in the rat. The vasoconstrictor action of these substances may be mediated by either a direct effect on renal vasculature (194) or through a stimulatory effect on renin release (25,26, Fig. 1.4).

From the preceding chapter, it will be remembered that inhibition of angiotensin II formation, by use of the angiotensin converting enzyme inhibitor, captopril, restored the increased renal vascular resistance of oestrogen treated WKY and SHR to the pretreatment level. If prostaglandins were exerting a prominant vasoconstrictor action on the renal vasculature of the above animals, in which any effect of prostaglandins on renin release (and subsequent generation of angiotensin II) had been removed, one might have expected that the renal vascular resistance of these animals would have remained at least somewhat elevated compared to their non-oestrogen treated controls. This would be especially so, given the stimulatory effect of captopril on prostaglandin production in man (195, 196) and more relevantly, the glomeruli of the rat (197). This was not the case however, indicating that the vasoconstrictor action of prostaglandins reported in this study, was not the result of a direct effect of these substances. On the other hand, there is a growing body of evidence to indicate that in the healthy condition, and under certain diseased states, prostaglandin biosynthesis may be

central to the control of renin release. This topic has been extensively reviewed by Jackson, et al (198). Of particular interest to this study are the reports which have shown that the administration of indomethacin can affect a reduction in blood pressure under conditions in which arterial pressure is maintained, in part, by elevated levels of plasma renin activity. This effect of indomethacin is associated with a reduction in plasma renin activity (27,199,200). Although plasma renin activity was not measured in the present study, the observation from Chapter 6, that both oestrogen treated WKY and SHR failed to suppress plasma renin concentration in the presence of an elevated plasma renin activity is consistent with a stimulatory effect of prostaglandins on renin release in the oestrogen treated rat.

Taken together, the findings described above suggest that a prostaglandin-dependant pathway is involved, in part, in the angiotensin II-mediated reduction in renal blood flow which has been identified in both oestrogen treated WKY and SHR, by the experiments described in the preceding chapter.

In conclusion, inhibition of prostaglandin synthesis has been found to have no effect on the arterial pressure of oestrogen treated SHR. This finding fails to support other evidence which indicates that prostaglandin-mediated vasodilation of peripheral resistance vessels is responsible for a reduction in arterial pressure in the SHR after oestrogen. However, differences in the indomethacin administration protocol between these sets of data may go some way to explain the apparently conflicting findings.

Prostaglandin synthesis inhibition has also been found to result in a reduction in renal vascular resistance in oestrogen treated WKY and SHR. It has been suggested that this apparent

renal vasoconstrictor action of prostaglandins was, in fact, mediated via a stimulatory effect of prostaglandins on renin secretion. A prostaglandin dependant pathway may therefore contribute to the angiotensin II-mediated constriction of the renal vasculature which has been identified in both WKY and SHR after oestrogen treatment.

Chapter 8

THE INFLUENCE OF DIETARY SODIUM RESTRICTION ON RENAL AND SYSTEMIC HAEMODYNAMICS IN RATS RECEIVING INJECTIONS OF OESTROGEN

8.1 INTRODUCTION

The reciprocal relationship between sodium intake and renin secretion is well documented (132,133). An alteration in dietary sodium intake is therefore a commonly used method of changing renin-angiotensin activity. This manoeuvre alters the transport of sodium in the distal tubule of the nephron and thus renin release (134). Dietary sodium restriction results in a marked increase in plasma renin activity (135). Conversely excessive sodium intake suppresses plasma renin activity (136).

In spite of causing an increased plasma renin activity, dietary sodium restriction characteristically has no major effect on either arterial pressure or renal blood flow in the normotensive state, although a reduction in both arterial pressure (137) and renal blood flow (138) have been recorded. There is, however, clear evidence demonstrating the dependancy of these haemodynamic variables, on the endogenous level of angiotensin II, in the salt-deplete state. This evidence is based on the reports that pharmacological interruption of the renin-angiotensin system, in salt deprived animals, results in a fall in arterial pressure (138, 139) and a rise in renal blood flow (138-140).

Although there is a body of data associating excess salt intake with hypertension (141), definitive proof is, at the present time lacking. In this context, dietary sodium restriction has been useful in reducing the blood pressure of hypertensive subjects (142). Recently however, the merits of the marginal hypotensive effect of this manoeuvre have been questioned (143).

The objective of this study therefore, was to investigate the effect of the chronic physiological challenge of dietary

sodium restriction - on renal and systemic haemodynamics - in rats receiving oestrogen. This approach presents an opportunity to assess the effectiveness of the control of the renal circulation when challenged by a combination of two manoeuvres, both capable of stimulating the renin-angiotensin system. The findings of this study may also help to define the benefits, if any, of a low sodium diet, to women who are susceptible to oestrogen-associated hypertension.

8.2 METHODS

Adult female Sprague-Dawley rats (Nottingham University: 190 - 240g), housed at 21 ± 1°C, were used throughout the study. Animals were fed either a normal sodium (0.34%) or low sodium (0.007%) diet (SDS, England), and drank tap water and distilled deionised water ad libitim, respectively. Oestrone acetate in ethyl oleate (4 mg per ml) was used to give subcutaneous injections of 1 mg oestrogen on alternate days. Oestrogen treatment and dietary sodium restriction were applied to five experimental groups of rats, according to the following schedule:

Experimental Group	Duration of Oestrogen Treatment - days	Type and Duration of Diet - days
1	-	Normal Na - 10
2	10	Normal Na - 10
3	20	Normal Na - 20
4	-	Low Na - 10
5	20	Normal Na - 10 followed by Low Na - 10

The control animals for oestrogen treated groups 2 and 3 were maintained on the normal sodium diet, and received the appropriate injection of vehicle alone.

As in preceding chapters, the injection of vehicle alone did not alter any of the parameters under study (Tables 8.1 and 8.2). Data from the vehicle-injected animals and group 1 have therefore been pooled, and form the control group for the experimental groups 2,3 and 4. The effect of dietary sodium restriction on oestrogen treated animals has been assessed by comparing groups 3 and 5.

24-hours after completing the schedule, animals were prepared for experimental analysis. Haemodynamic variables, renin-angiotensin system parameters (including both trypsin- and acid-activable renin) and the packed cell volume of blood samples were measured as described in previous chapters.

8.3 RESULTS

Effect of Oestrogen Treatment

Haemodynamics (Tables 8.3 and 8.4)

Mean arterial pressure was elevated after 10 days of oestrogen treatment ($p < 0.05$), with no significant changes in either cardiac output or total peripheral resistance. Arterial pressure increased further after 20 days of oestrogen ($p < 0.001$): this pressure being significantly greater than that recorded after 10 days of oestrogen ($p < 0.05$). Whilst cardiac output remained unchanged after 20 days of oestrogen, total peripheral resistance was elevated ($p < 0.01$).

Whereas renal vascular resistance was unchanged after 10 days of oestrogen, it became elevated after 20 days of treatment ($p < 0.05$). Renal blood flow was not affected by oestrogen.

Active Renin (Tables 8.5 and 8.6)

Plasma renin activity was unchanged after 10 days of oestrogen, but was increased after 20 days ($p < 0.01$).

Plasma and renal renin concentrations were decreased, both after 10 ($p < 0.01$) and 20 days ($p < 0.001$) of oestrogen treatment.

Inactive Renin (Tables 8.5 - 8.7)

Inactive plasma renin was increased after both 10 ($p < 0.001$) and 20 days ($p < 0.05$) of oestrogen treatment.

Inactive renal renin was unchanged after 10 days of oestrogen, and decreased after 20 days of treatment ($p < 0.05$).

Packed Cell Volume (Tables 8.3 and 8.4)

The packed cell volume was unchanged after 10 days of oestrogen, but decreased after 20 days of treatment ($p < 0.001$).

In summary, a rise in mean arterial pressure was noted in rats after 20 days of oestrogen treatment. This rise was accompanied by an increase in both total peripheral resistance and plasma renin activity, and a fall in the packed cell volume. Oestrogen treatment had no effect on renal blood flow. An increase in circulating inactive renin was observed in oestrogen treated rats.

Effect of Dietary Sodium Restriction

Haemodynamics (Tables 8.3 and 8.4)

10 days of dietary sodium restriction reduced mean arterial pressure ($p < 0.01$). Sodium depletion reduced cardiac output ($p < 0.05$), but had no effect on total peripheral resistance.

Neither renal blood flow or renal vascular resistance were influenced by dietary sodium restriction.

Active Renin (Tables 8.5 and 8.6)

Whilst plasma renin activity increased with sodium restriction ($p < 0.001$), both plasma and renal renin concentrations remained unchanged.

Inactive Renin (Tables 8.5 - 8.7)

No inactive renin was detected in the plasma of rats fed the low sodium diet. Inactive renal renin was not affected by

sodium restriction.

Packed Cell Volume (Tables 8.3 and 8.4)

Dietary sodium restriction did not affect the packed cell volume.

In summary, 10 days of dietary sodium restriction resulted in a marked rise in plasma renin activity. In spite of this rise, mean arterial pressure fell, due to a reduced cardiac output. Renal blood flow was not affected by sodium depletion. No inactive renin was detected in the plasma of sodium deprived rats.

Effect of Dietary Sodium Restriction in Rats Receiving Oestrogen

Haemodynamics (Tables 8.3 and 8.4 and Figure 8.1)

Sodium restriction decreased the mean arterial pressure of oestrogen treated rats ($p < 0.05$). The arterial pressure of these rats was significantly larger than the non-oestrogen treated control value ($p < 0.05$), but similar to the pressure noted in rats after 10 days of oestrogen treatment. Cardiac output was increased ($p < 0.05$) and total peripheral resistance reduced ($p < 0.01$) in sodium restricted oestrogen treated rats.

Sodium deprivation had no effect on either the renal blood flow or renal vascular resistance of oestrogen treated rats.

Active Renin (Tables 8.5 and 8.6)

Neither the plasma renin activity nor the plasma and renal renin concentrations of oestrogen treated rats were influenced by sodium restriction.

Inactive Renin (Tables 8.5 - 8.7)

No inactive renin was detected in either plasma or renal cortical tissue of oestrogen treated rats fed the low salt diet.

Packed Cell Volume (Tables 8.3 and 8.4)

Whilst sodium restriction increased the packed cell volume of oestrogen treated rats ($p < 0.05$), the packed cell volume of these animals was significantly smaller than the value noted in non-oestrogen treated control rats ($p < 0.001$).

In summary, dietary sodium restriction affected a marginal fall in mean arterial pressure and a small increase in the packed cell volume of oestrogen treated rats. The renal blood flow of oestrogen treated rats was not affected by sodium restriction. Whilst sodium deprivation did not affect the plasma renin activity of oestrogen treated rats, it eliminated inactive renin from the plasma of these animals.

8.4 DISCUSSION

The findings from this study have shown that 10 days of dietary sodium restriction had no effect on the renal blood flow of rats undergoing a 20 day period of oestrogen treatment. This sodium restriction did, however, prevent an exacerbation of the hypertension noted in oestrogen treated rats. This effect was associated with an attenuation of a contracting packed cell volume. Whereas sodium restriction affected a marked rise in plasma renin activity in non treated rats, it had no influence on the plasma renin activity of oestrogen treated animals. No inactive renin could be detected in the plasma of both non treated and oestrogen treated rats fed the low sodium diet.

Dietary sodium restriction did not affect either the level of renal perfusion, or the autoregulation of renal blood flow in response to a fall in perfusion pressure - in both non treated and oestrogen treated rats. Similarly, oestrogen treatment had no effect on renal blood flow. This latter observation is in contrast to the findings from chapter 3, where a fall in renal

TABLE 8.1. Effect of Vehicle (ethyl oleate) Injection on Haemodynamics and the Packed Cell Volume.

Data are presented as either a mean ± 95% confidence limits or a median and range. In the test column an F value indicates the use of a one way ANOVA; a Z value - the use of the Wilcoxon Rank Sum test for two groups, and an H value - the Krumkal-Wallis test for three groups.

PARAMETER	NON TREATED	CONTROL FOR GROUP 2	CONTROL FOR GROUP 3	TEST
Mean Arterial Pressure mm Hg	117±4 n=9	120±3 n=8	122±7 n=8	F = 0.76 NS
Heart Rate beats per min	397±20 n=5	394±24 n=8	395±10 n=8	F = 0.03 NS
Cardiac Output ml min^{-1} kg^{-1}	240±25 n=9	283±33 n=8	254±33 n=8	F = 1.98 NS
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.570±0.040 n=5	0.720±0.100 n=8	0.640±0.080 n=8	F = 3.03 NS
Total Peripheral Resistance dyn cm^{-5} sec^{-1} kg^{-1}	39,890±4,416 n=9	34,772±4,500 n=8	40,061±7,430 n=8	F = 1.10 NS
Renal Blood Flow ml min^{-1} g^{-1}	5.35±0.82 n=9	5.77±0.73 n=8	5.47±0.49 n=8	F = 0.35 NS
Renal Vascular Resistance dyn cm^{-5} sec^{-1} kg^{-1}	1,743: 1,315-3,154 n=9	1,709±220 n=8	1,804±178 n=8	H = 1.28 NS
Packed Cell Volume %	37.9±1.6 n=5	36.1±0.6 n=8	35.7±0.8 n=8	F = 4.61 NS

TABLE 8.2. Effect of Vehicle (ethyl oleate) Injection on Inactive and Active Renin.

Legend as described in Table 8.1

PARAMETER	NON TREATED	CONTROL FOR GROUP 2	CONTROL FOR GROUP 3	TEST
Plasma Renin Activity ng ang I ml^{-1} hr^{-1}	7.88±0.69 n=5	6.51±1.20 n=8	9.56±2.45 n=8	F = 3.01 NS
Total Renin Activity ng ang I ml^{-1} hr^{-1}	-	7.43±0.98 n=8	10.15±2.76 n=8	F = 2.47 NS
Inactive Renin ng ang I ml^{-1} hr^{-1}	-	0.92±0.43 n=8	0.11: n=8 0 - 2.17	Z = -1.36 NS
Inactive Renin % total renin activity	-	13.06±6.31 n=8	4.92±4.47 n=8	F = 4.25 NS
Plasma Renin Concentration ng ang I ml^{-1} hr^{-1}	97.90±26.07 n=5	89.8±19.0 n=8	106.8±15.9 n=8	F = 0.83 NS
Plasma Renin Substrate Concentration ng per ml	230±16 n=4	-	-	-
Renal Renin Concentration ng ang I mg^{-1} hr^{-1}	11,192±2,435 n=4	9,418±2,315 n=7	12,166±1,931 n=8	F = 1.73 NS
Renal Renin Concentration (dialysed control) ng ang I mg^{-1} hr^{-1}	13,006±3,042 n=4	9,012±1,605 n=7	12,268±1,799 n=8	F = 1.77 NS
Total Renin concentration ng ang I mg^{-1} hr^{-1}	14,530±4,318 n=4	9,778±1,793 n=7	13,728±2,043 n=8	F = 1.95 NS
Inactive Renal Renin ng ang I mg^{-1} hr^{-1}	1,456±1,076 n=4	766±508 n=7	1,459±670 n=8	F = 1.20 NS
Inactive Renal Renin % total renin concentration	10.52±3.67 n=4	7.68±4.47 n=7	10.36±4.39 n=8	F = 0.51 NS

TABLE 8.3. Effect of Oestrogen Treatment and Dietary Sodium Restriction on Haemodynamic Parameters and the Packed Cell Volume.

Data are presented as means ± 95% C.L. Group 2 and Groups 3 and 5 received 1 mg oestrone acetate on alternate days for 10 and 20 days respectively. Controls and Groups 2 and 3 were fed a normal sodium diet. Group 4 received a low sodium diet for 10 days. Group 5 rats were fed the normal sodium diet for the first 10 days of oestrogen treatment and the low sodium diet for the last 10 days of treatment. * - $p<0.05$: ** - $p<0.01$: *** - $p<0.001$ - significantly different from controls

PARAMETER	CONTROLS	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Mean Arterial Pressure mm Hg	119±3 n=25	*128±6 n=9	***137±6 n=9	**111±6 n=14	127±6 n=10
Heart Rate beats per min	395±10 n=21	**365±18 n=9	*373±18 n=9	371±27 n=14	316±31 n=10
Cardiac Output ml min^{-1} kg^{-1}	258±18 n=25	233±24 n=9	234±37 n=9	*202±57 n=7	294±31 n=9
Stroke Volume ml $beat^{-1}$ kg^{-1}	0.65±0.05 n=21	0.65±0.07 n=9	0.63±0.10 n=9	0.61 0.16 n=7	0.95±0.12 n=9
Total Peripheral Resistance dyn cm^{-5} sec^{-1} kg^{-1}	38,307±3,212 n=25	43,622±5,057 n=9	**49,377±8,416 n=9	45,019±10,984 n=7	34,906±3,630 n=9
Renal Blood Flow ml min^{-1} g^{-1}	5.52±0.39 n=25	5.44±0.86 n=9	5.11±0.35 n=9	5.54±1.35 n=7	5.28±0.92 n=9
Renal Vascular Resistance dyn cm^{-5} sec^{-1} kg^{-1}	1,803±172 n=25	1,806±186 n=9	*2,169±198 n=9	1,606±361 n=7	*2,054±455 n=9
Packed Cell Volume %	36.40±0.63 n=21	35.4±1.2 n=8	***32.2±0.8 n=9	36.3±2.4 n=7	---33.7±0.57 n=9

TABLE 8.4. Statistical Analysis of the Effect of Oestrogen Treatment and Dietary Sodium Restriction on Haemodynamic Parameters and the Packed Cell Volume. Experimental groups are as described in Table 5.1. Data have been analysed using a one way ANOVA. * - $p<0.05$: ** - $p<0.01$: *** - $p<0.001$: NS - not significant. Asterisks denote the level of significance of the variance ratio - F.

PARAMETER	CONTROLS v. GROUP 2	CONTROLS v. GROUP 3	CONTROLS v. GROUP 4	CONTROLS v. GROUP 5	GROUP 2 v. GROUP 3	GROUP 3 v. GROUP 5
Mean Arterial Pressure	F = 7.26 *	F = 30.70 ***	F = 8.39 **	F = 6.10 *	F = 5.37 *	F = 5.46 *
Heart Rate	F = 8.92 **	F = 5.00 *	F = 3.55 NS	F = 34.07 ***	F = 0.37 NS	F = 8.57 **
Cardiac Output	F 2.17 NS	F = 1.67 NS	F = 6.05 *	F = 3.78 NS	F = 0 NS	F = 5.87 *
Stroke Volume	F = 0 NS	F = 0.27 NS	F = 0.37 NS	F = 18.15 ***	F = 0.16 NS	F = 16.30 ***
Total Peripheral Resistance	F = 2.67 NS	F = 8.83 **	F = 2.52 NS	F = 1.32 NS	F = 1.2 NS	F = 9.58 **
Renal Blood Flow	F = 0.04 NS	F = 1.38 NS	F = 0 NS	F = 0.32 NS	F = 0.49 NS	F = 0.11 NS
Renal Vascular Resistance	F = 0 NS	F = 5.25 *	F = 1.04 NS	F = 1.57 NS	F = 6.74 *	F = 0.21 NS
Packed Cell Volume	F = 2.49 NS	F = 36.94 ***	F = 0 NS	F = 29.13 ***	F = 17.21 ***	F = 8.20 *

TABLE 8.5. Effect of Oestrogen Treatment and Dietary Sodium Restriction on Inactive and Active Renin.
Legend as described in Table 8.3.

PARAMETER	CONTROLS	GROUP 2	GROUP 3	GROUP 4	GROUP 5
Plasma Renin Activity ng ang I ml^{-1} hr^{-1}	8.00±1.18 n=21	6.33±1.78 n= 9	** 11.69±2.12 n=11	*** 17.26±4.33 n=8	9.7±2.35 n=10
Total Renin Activity ng ang I ml^{-1} hr^{-1}	8.61±1.59 n=16	9.77±1.69 n= 9	*** 14.02±1.47 n=11	*** 16.37±3.06 n=8	9.52±2.69 n=10
Inactive Renin ng ang I ml^{-1} hr^{-1}	0.75±0.39 n=16	*** 3.44±1.63 n= 9	* 2.33±1.35 n=11	0 n=8	0 n=10
Inactive Renin % Total renin activity	8.99±4.27 n=16	*** 34.53±14.67 n= 9	17.58±9.47 n=11	0 n=8	0 n=10
Plasma Renin Concentration ng ang I ml^{-1} hr^{-1}	98.2±11.2 n=21	** 60.7±25.9 n= 8	*** 34.5±5.5 n= 8	105.3±43.7 n=8	*** 28.4±8.0 n=10
Plasma Renin Substrate Concentration - ng per ml	230±16 n= 4	** 424±69 n= 8	*** 611±78 n= 8	214±23 n=8	—
Renal Renin Concentration ng ang I mg^{-1} hr^{-1}	10,948±1,341 n=19	*** 6,333±1,590 n= 8	*** 4,915±1,060 n=11	10,761±888 n=8	*** 3,905±566 n=10
Renal Renin Concentration (dialysed control) ng ang I mg^{-1} hr^{-1}	10,754±1,523 n=20	** 6,975±1,480 n=10	*** 5,190±1,054 n=11	11,417±1,064 n=8	*** 4,302±768 n=10
Total Renin Concentration ng ang I mg^{-1} hr^{-1}	11,957±1,801 n=20	* 8,256±1,993 n=10	*** 5,652±1,080 n=11	12,329±1,574 n=8	*** 4,511±690 n=10
Inactive Renal Renin ng ang I mg^{-1} hr^{-1}	1,216±421 n=20	1,282±600 n=10	* 447±316 n=11	987±602 n=8	0 n=10
Inactive Renal Renin % Total renin concentration	9.46±2.47 n=20	14.08±4.29 n=10	8.89±5.80 n=11	7.34±3.78 n=8	0 n=10

TABLE 8.6. Statistical Analysis of the Effect of Oestrogen Treatment and Dietary Sodium Restriction on Inactive and Active Renin. Legend as described in Table 8.4.

PARAMETER	CONTROLS v. GROUP 2	CONTROLS v. GROUP 3	CONTROLS v. GROUP 4	CONTROLS v. GROUP 5	GROUP 2 v. GROUP 3	GROUP 3 v. GROUP 5
Plasma Renin Activity	F= 2.36 NS	F=10.58 **	F=31.56 ***	F= 2.19 NS	F=13.53 ***	F=1.42 NS
Total Renin Activity	F= 0.77 NS	F=21.44 ***	F=24.01 ***	F= 0.38 NS	F=14.40 ***	F=8.56 **
Inactive Renin ng ang I ml^{-1} hr^{-1}	F=16.39 ***	F= 6.56 *	—	—	F= 1.09 NS	—
Inactive Renin %	F=16.73 ***	F= 3.25 NS	—	—	F= 3.88 NS	—
Plasma Renin Concentration	F= 9.31 **	F=44.57 ***	F= 0.19 NS	F=62.63 ***	F= 3.77 NS	F=1.37 NS
Plasma Renin Substrate Concentration	F=14.53 ***	F=43.58 ***	F= 0.56 NS	—	F=12.46 **	—
Renal Renin Concentration	F=15.23 ***	F=36.94 ***	F= 0.03 NS	F=52.39 ***	F= 2.29 NS	F=2.55 NS
Renal Renin Concentration (dialysed control)	F= 9.50 **	F=24.39 ***	F= 0.27 NS	F=31.14 ***	F= 3.82 NS	F=1.42 NS
Total Renin Concentration	F= 6.43 *	F=23.11 ***	F= 0.06 NS	F=31.18 ***	F= 4.84 *	F=2.91 NS
Inactive Renal Renin ng ang I mg^{-1} hr^{-1}	F= 0.03 NS	F= 5.50 *	F= 0.34 NS	Z=-3.04 **	F= 5.73 *	—
Inactive Renal Renin %	F= 3.84 NS	F= 0.05 NS	F= 0.82 NS	Z=-1.80 *	F= 1.95 NS	—

TABLE 8.7. Statistical Analysis of the Effect of Trypsin Treatment and Acidification on Plasma Renin Activity and Renal Renin Concentration Respectively.

Controls and experimental groups 2 - 5 are as described in Table 5.3. The difference between non treated and trypsin or acid treated groups has been analysed using a paired t - test. Asterisks denote the level of significance of the test statistic -T. NS = non significant.

	NON TREATED GROUP	TRYPSIN OR ACID TREATED GROUP	MEAN DIFFERENCE BETWEEN GROUPS	N	TEST
CONTROLS	PRA	TRA	- 0.57	16	T = -2.23 *
	RRC	TRC	- 1,203	20	T = -5.50 ***
GROUP 2	PRA	TRA	- 3.38	9	T = -3.91 ***
	RRC	TRC	- 1,191	10	T = -3.34 **
GROUP 3	PRA	TRA	- 2.27	11	T = -3.19 **
	RRC	TRC	- 462	11	T = -2.78 *
GROUP 4	PRA	TRA	0.88	8	T = 1.15 NS
	RRC	TRC	-912	8	T = -2.62 *
GROUP 5	PRA	TRA	0.24	10	T = 0.61 NS
	RRC	TRC	-128	10	T = -0.89 NS

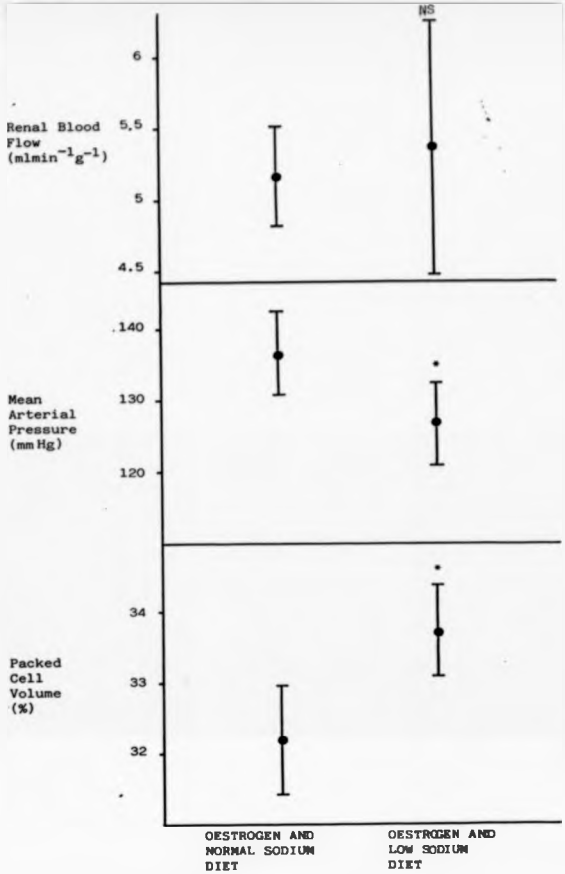


Figure 8.1 The Effects of Dietary Sodium Restriction on the Renal Blood Flow, Mean Arterial Pressure and Packed Cell Volume of Oestrogen Treated Rats. Both groups of rats received 1mg oestrone acetate on alternate days for 20 days. The low sodium diet group of rats were fed a low sodium diet for the latter 10 days of oestrogen treatment.

blood flow with oestrogen was noted in group B (10 mg oestrone over a period of either 10 or 20 days). However, out of necessity, different oestrogen dosing regimes have been employed in these two studies.

In non-oestrogen treated rats, dietary sodium restriction resulted in a reduction in arterial pressure, due to a fall in cardiac output. These findings are in agreement with published data concerning the rat (144,145). The fall in arterial pressure mentioned above may have contributed to the marked increase in plasma renin activity which was noted in these animals. It was of interest to note that in spite of the changes mentioned above, the packed cell volume of non-oestrogen treated rats fed the low salt diet was normal suggesting that this manoeuvre had, if anything, only a minimal effect on extracellular fluid volume in these animals.

In oestrogen treated rats, dietary sodium restriction prevented the rise in blood pressure which took place during the latter 10 days of oestrogen treatment. This effect of sodium deprivation was associated with an attenuation of a contraction in the packed cell volume. The arterial pressure and packed cell volume of oestrogen treated rats fed the low salt diet were, however, significantly larger and smaller respectively, than the values recorded for these variables in non-oestrogen treated rats. Taken together, these data support the hypothesis that the oestrogen-induced hypertension encountered in this investigation is mediated in part by a powerful antinatriuretic action of oestrogen, which in turn leads to fluid retention and arterial overfill. Further, whilst a low sodium diet may be successful in preventing an exacerbation of oestrogen-associated hypertension, it would appear to be of only minimal benefit as a form of antihypertensive therapy.

The present findings clearly show that whereas dietary sodium restriction caused a marked rise in the plasma renin activity of non treated rats, it had no effect on the plasma renin activity of oestrogen treated animals. Oestrogen and sodium restriction stimulate plasma renin activity by different pathways - oestrogen increases renin substrate synthesis (39) and sodium restriction increases renin release (134). The former pathway initiates a powerful negative feedback inhibition on renin secretion, as evidenced by a marked reduction in both plasma and renal renin concentrations in all the oestrogen treated groups of rats used in this study. This effect of oestrogen may explain the failure of sodium restriction to cause an increase in plasma renin activity in the oestrogen treated rat.

Inactive renin was absent from the plasma of both groups of rats which were fed the low sodium diet. In non-oestrogen treated rats a marked rise in plasma renin activity was noted in association with the disappearance of plasma inactive renin. This observation is in agreement with the findings from the preceding chapter which, it was suggested, indicate that inactive plasma renin is a direct precursor of active plasma renin. Moreover, there is evidence to indicate that conversion of inactive to active plasma renin is a physiological event, mediated intrarenally (119) by kallikrein (108).

In the salt-deplete state, it will be remembered, renal perfusion is highly dependant on angiotensin II (140). In this situation, enhanced intrarenal production of both kallikrein (28) and prostaglandins (27) antagonise the pressor action of angiotensin II, and thereby serve to modulate renal

haemodynamics. In the sodium-deplete state therefore, circulating inactive renin may represent a significant post-secretary source of renin, which is recruited intrarenally by kallikrein, in order to maintain renal perfusion through the pressor action of angiotensin II. Consistent with this hypothesis is the absence of inactive renin in the plasma of rats fed the low sodium diet.

In conclusion, although both oestrogen treatment and dietary sodium restriction were found to increase plasma renin activity in the rat when administered independantly, renal haemodynamics were found to be normal in a group of oestrogen treated rats fed a low sodium diet. Further, sodium restriction had only a marginal effect on the hypertension noted in oestrogen treated rats.

Chapter 9

GENERAL DISCUSSION

9.1 RESUME OF THE BACKGROUND AND PURPOSE OF INVESTIGATION

A healthy renal circulation is critical to normal renal function. Control of the renal circulation and the maintenance of cardiovascular homeostasis are linked through a number of common intrarenal pathways involving vasoactive compounds and extracellular fluid volume regulation. Oestrogens have the potential to influence the renal circulation (and thereby cardiovascular function) - principally through their effects on blood pressure, vasoactive compounds - particularly angiotensin II, and smooth muscle receptor physiology. In spite of this potential, and the widespread use of oestrogens for contraceptive purposes, the effect of these steroids on the renal circulation has received only minimal attention.

This investigation was undertaken with the objective of assessing the connection between oestrogen-induced changes in renal and systemic haemodynamics, and the relationship of these changes to alterations in intrarenal vasoactive compounds and plasma volume status. This multifaceted approach has been achieved by the use of radiolabelled microspheres and radioimmunoassay to measure haemodynamic and renin-angiotensin variables respectively, tryptic and acid activation of inactive renin, dietary sodium restriction and inhibition of both angiotensin II formation and prostaglandin synthesis. Both normotensive and spontaneously hypertensive strains of rat have been studied.

It need be emphasised that no measurements of circulating oestrone concentrations have been made during this investigation. The amounts of oestrone which have been used are pharmacological, and larger than the doses of other types of oestrogens commonly used by most workers. The relative impotency of oestrone compared to

these other oestrogens (39,201) would, however, be likely to result in comparable levels of oestrogenic activity.

9.2 GENERAL DISCUSSION

The findings from chapter 3 of this investigation have demonstrated that oestrogen can cause a reduction in renal blood flow and an increase in arterial pressure in the normotensive rat. Whilst these haemodynamic changes took place independently of one another, they were accompanied by marked alterations in both the renin-angiotensin system and plasma volume status.

The reduction in renal blood flow described above was due to an increased renal vascular resistance in the presence of an elevated plasma renin activity. This latter change - which probably occurred as the result of a profound increase in renin substrate synthesis - suggests that angiotensin II-mediated constriction of the renal vasculature was responsible for the increased renal vascular resistance. Strong support for this hypothesis was provided by the findings from chapter 7. Here, administration of the angiotensin converting enzyme inhibitor, captopril, to WKY rats with an oestrogen-induced reduction in renal blood flow, was found to restore renal blood flow to the pretreatment level - by reducing renal vascular resistance.

The angiotensin II-mediated vasoconstriction described above may have occurred as the result of a general increase in the concentration of angiotensin II throughout the circulation, as indicated by the observed increased plasma renin activity. However, whilst it was found that changes in plasma renin activity were reciprocally related to changes in renal blood flow in rats with a normal level of renal perfusion, no such relationship was noted

in rats with an oestrogen-induced reduction in renal perfusion. This observation, in conjunction with the fact that oestrogen-induced increases in renal vascular resistance were much greater than those noted in peripheral vascular resistance, suggests that there is a critical intrarenal focus for the action of angiotensin II in the oestrogen treated rat. This hypothesis is in keeping with the concept of angiotensin II as a local renal vascular hormone (9-11). Angiotensin II is, however, only a single element in an intrarenal hormonal complex, which includes prostaglandins and kinins, and which is concerned with the modulation of renal haemodynamics (12,13,16,19,27,28). This investigation provides evidence to suggest that oestrogen can disturb the normal equilibrium which exists between these intrarenal vasoactive compounds (27,28). This effect ultimately leads to inappropriate, angiotensin II-mediated constriction of the renal vasculature, thereby reducing renal perfusion.

The findings from chapter 4 are consistent with the presence of an inactive form of renin in rat plasma - which is of renal origin. The inverse relationship which has been recorded between inactive and active renin supports the concept that inactive renin is a proenzyme precursor for active renin. There is evidence to suggest that conversion of inactive to active renin is a physiological event (119), mediated intrarenally by Kallikrein (108). Inactive renin in plasma may therefore represent a significant postsecretory source of renin, which might serve to regulate the amount of angiotensin II available to the renal circulation. Oestrogens have been reported to activate the kallikrein-kinin system (42,43). This observation, coupled with the finding that inactive renin was absent from the plasma of rats with an oestrogen-induced reduction in renal blood flow, suggests

that conversion of active to inactive renin was enhanced in these animals. In the presence of an elevated concentration of renin substrate, the additional intrarenal angiotensin II thus generated would be likely to increase renal vascular resistance. The findings from a preliminary study designed to test the above hypothesis indicate that acute inhibition of renal kallikrein activity, through use of the serine esterase inhibitor, aprotinin, did not alter either the renal blood flow or arterial pressure of oestrogen-treated normotensive rats.

The findings from chapter 7 have reported an apparently intrarenal vasoconstrictor mode of action by renal prostaglandins, in both WKY and SHR after oestrogen. Here, inhibition of prostaglandin synthesis, by administration of indomethacin, resulted in an increase in renal blood flow, through a decrease in renal vascular resistance. This effect of renal prostaglandins was, however, probably mediated via a stimulatory action of prostaglandins on renin release (and subsequent generation of angiotensin II) (198). Support for this hypothesis may be derived from the observation that the renal vascular resistance of oestrogen treated WKY and SHR given captopril was not even slightly elevated compared to pretreatment values. Thus a prostaglandin-dependant pathway might contribute in part to the angiotensin II-mediated renal vasoconstriction identified in both WKY and SHR after oestrogen.

The experiment described in chapter 5 was designed to evaluate the role of angiotensin II in oestrogen-induced haemodynamic disturbances, through use of the angiotensin II antagonist - saralasin. However, the antagonist was found to exhibit agonistic-like activity in oestrogen treated rats, with a particularly marked expression on the renal vasculature. Whereas this agonistic activity did not permit any conclusions in connection with the primary objective of the experiment, it suggests that oestrogen may have an influence on vascular angiotensin II receptor physiology in these animals. This proposal is based on the

reports which have shown that the agonistic property of saralasin is prominent in states in which the density of vascular angiotensin II receptors is elevated (150, 154, 162). Oestrogens can increase the number of angiotensin II receptors, both in the glomeruli of normotensive rats (A. Messenger: personal communication) and in the myometrium (47). Thus an increase in the density of intrarenal vascular angiotensin II receptors with oestrone would, in the presence of an increased level of endogenous angiotensin II, be likely to increase renal vascular resistance.

Collectively, the findings from this investigation demonstrate that the reduction in renal blood flow noted in the normotensive rat after oestrogen can be attributed to angiotensin II-mediated constriction of the renal vasculature, which was principally due to a marked stimulation of renin substrate synthesis. Both prostaglandin- and kallikrein-dependant pathways may contribute to this angiotensin II-mediated vasoconstriction, which might be magnified in the presence of an increase in the density of intrarenal vascular angiotensin II receptors.

The rise in arterial pressure described at the beginning of this discussion took place without any marked alteration in either cardiac output or total peripheral resistance. This observation suggests that both 'volume' and 'vasoconstrictor' factors were involved in the maintenance of this hypertension.

Throughout this investigation oestrogen treatment has been found to consistently reduce the packed cell volume. This reduction, indicative of plasma volume expansion, was probably mediated by the salt and water retaining properties of oestrogen (91-93). Moreover, the observation that changes in the packed cell volume

were reciprocally related to changes in mean arterial pressure is consistent with a prominent volume component in the mediation of hypertension. The findings from chapter 5 have demonstrated that dietary sodium restriction had only a minimal effect on the packed cell volume of oestrogen treated rats, and was therefore of marginal benefit as a form of antihypertensive therapy in these animals.

The involvement of angiotensin II in the maintenance of the oestrogen-induced hypertension reported in this investigation was demonstrated by the findings from chapter 6. Here, administration of captopril to WKY rats with an increase in arterial pressure, due to oestrogen pretreatment, was found to reduce arterial pressure.

Taken together, the findings from this investigation indicate that the rise in mean arterial pressure noted in the normotensive rat after oestrogen, was determined by a combination of plasma volume expansion and a renin-angiotensin activity which was inappropriately high in relation to plasma volume status.

The findings from chapter 7 of this investigation have demonstrated that oestrone also has a pronounced effect on renal and systemic haemodynamics, the renin-angiotensin system and plasma volume status in the SHR. Like its normotensive WKY counterpart, the SHR exhibited a reduced renal blood flow after oestrogen treatment. Unlike WKY however, the mean arterial pressure of SHR dropped after oestrogen. This latter finding is in contrast to the reported exacerbation of pre-existing hypertension in women ingesting oestrogen-containing oral contraceptives (165,166). This observation raises the possibility of an important difference in the circulatory response to oestrogen, between the most widely

used experimental animal model of human essential hypertension, and individuals manifesting the disease itself.

The drop in mean arterial pressure, noted in SHR after oestrogen, took place in spite of a reduction in the packed cell volume, and increases in cardiac output, renal vascular resistance and plasma renin activity - all of which might be expected to promote an increase in arterial pressure. A marked reduction in peripheral vascular resistance was therefore responsible for this fall in perfusion pressure. These findings suggest that vasodilation of peripheral resistance vessels in the presence of a diminished vascular responsiveness to angiotensin II was responsible for the drop in arterial pressure. This vasodilation may have been mediated by a stimulatory effect of oestrogen on vascular prostaglandin synthesis (44-46). Support for the above hypothesis may be derived from the reported abnormalities in the responsiveness of the renin-angiotensin system in both the SHR(182) and essentially hypertensive humans (179-181), the increased responsiveness to vasodepressor prostaglandins exhibited by SHR (184), and the observation that chronic indomethacin administration can prevent a fall in arterial pressure in the oestrogen treated SHR (C. Stoner: personal communication). The findings from chapter 7 of this investigation however, fail to support the aforementioned hypothesis. Here, acute administration of indomethacin to SHR pretreated with oestrogen, failed to affect an increase in the arterial pressure of these rats.

The proposed diminution of the responsiveness of peripheral resistance vessels to the vasoconstrictor action of angiotensin II, did not extend to the renal vasculature of the oestrogen treated SHR. Here, the vasconstrictor action of angiotensin II led to a marked increase in renal vascular resistance, which resulted in

a fall in renal blood flow. Thus, as for WKY, captopril restored the renal blood flow of oestrogen treated SHR by reducing renal vascular resistance.

On the basis of the haemodynamic changes reported in this investigation, it is evident that oestrone can disturb normal cardiovascular function in the rat. Moreover, it is clear that the renal circulation is a major site for this effect of oestrone. Here, a disturbance of the normal equilibrium between intrarenal vasoactive substances, in conjunction with an increase in the density of vascular angiotensin II receptors, may result in an enhanced local generation of angiotensin II. This angiotensin II has been shown to result in an inappropriate increase in renal vascular resistance, thereby reducing renal perfusion. The observations that oestrogens can cause both structural (50) and functional disturbances of the renal circulation in addition to their known influence on intrarenal vasoactive substances suggests that oestrogens may play a role, under certain circumstances, in either the initiation or maintenance of certain renal disorders in women. In this context, the increased incidence of autoimmune disease, particularly immune complex disease, seen in females (human and several animal species), may be related to hormonal status. Support for this view may be derived from the report of Friedman, et al (202). These workers have shown that oestrogen may affect Fc receptor-bearing cells that participate in the handling of immune complexes, thereby altering the clinical expression of the disease.

The primary objective of this investigation has been to contribute to a better comprehension of the little-understood subject of the involvement of intrarenal vasoactive substances

in the modulation of renal haemodynamics during oestrogen treatment. In the light of this investigation, there need be an increased awareness of a potentially harmful effect of oestrogen on the renal circulation, particularly in women ingesting oral contraceptives.

APPENDIX

Depth of Anaesthesia and Haemodynamic Measurements

The effect of anaesthesia on cardiovascular stability has been described in section 2.3.2 (p 18). In order to minimise this effect it is critical that a consistent depth of anaesthesia be attained at the point of measurement of haemodynamic variables. For the purpose of this study therefore, measurements of both mean arterial pressure and the remaining haemodynamic variables have been made at the lightest possible surgical plane of anaesthesia (as determined by pain and blink reflexes, and monitoring both blood pressure and the respiratory rhythm).

REFERENCES

1. Fourman, J. and Moffat, D.B. (1971). The Blood Vessels of the Kidney. Oxford, Blackwell

2. Folkow, B. (1964) Description of the Myogenic Hypothesis Circulation Research, 15, 279-287

3. Johnson, P.C. (1964) Review of Previous Studies and Current Theories of Autoregulation. Circulation Research, 15, 12-19.

4. Thurau, K. and Levine, D.Z. (1971) The Renal Circulation. In: Rouiller, C.L. and Muller, A.F. (eds.) The Kidney, 3, 2-70 New York, Academic Press.

5. Herbaczynska-Cedro, K. and Vane, J.R. (1973) Contribution of Intrarenal Generation of Prostaglandin to Autoregulation of Renal Blood Flow in the Dog. Circulation Research, 33, 428-436.

6. Mandel, M.J. and Sapirstein, L.A. (1962) Effect of Angiotensin Infusion on Regional Blood Flow and Regional Vascular Resistance in the Rat. Circulation Research, 10, 807-816.

7. Schmid, H.E. Jr. (1962) Renin, a Physiologic Regulator of Renal Haemodynamics. Circulation Research, 11, 185-193

8. Sokabe, H. (1974) Phylogeny of the Renal Effects of Angiotensin. Kidney International, 6, 263-271

9. Granger, P., Dahlheim, H. and Thurau, K. (1972) Enzyme Activities of the single Juxtaglomerular Apparatus in the Rat Kidney. Kidney International, 1, 78-88

10. Bailie, M.D., Rector, F.C. Jr. and Seldin, D.W. (1971) Angiotensin II in Arterial and Renal Venous Plasma and Renal Lymph in the Dog. Journal of Clinical Investigation, 50, 119-126

11. DiSalvo, J., Peterson, A., Montefusco, C. and Menta, M. (1971) Intrarenal Conversion of Angiotensin I to Angiotensin II in the Dog. Circulation Research, 29, 398-406

12. Cowley, A.W.Jr., Miller, J.P. and Guyton, A.C. (1971) Open-Loop Analysis of the Renin-Angiotensin System in the Dog. Circulation Research, 28, 268-281

13. Lohmeir, T.E., Cowley, A.W. Jr., Trippodo, N.C., Hall, J.E. and Guyton, A.C. (1977) Effects of Endogneous Angiotensin II on Renal Sodium Excretion and Renal Haemodynamics. American Journal of Physiology, 233, F388-F395

14. Hall, J.E., Guyton, A.C., Jackson, T.E., Coleman, T.G., Lohmeier, T.E. and Trippodo, N.C. (1977) Control of Glomerular Filtration Rate by Renin-Angiotensin System. American Journal of Physiology, 233, F366-F372

15. Johnston, H.H., Herzog, J.P. and Lauler, D.P. (1967) Effect of Prostaglandin E, on Renal Haemodynamics, Sodium and Water Excretion. American Journal of Physiology, 213, 939-946

16. Bolger, P.M., Fisher, G.M., Shea, P.T., Rasmuell P.W. and Slotkiff, L.M. (1977) Effects of PGD_2 on Canine Renal Function. Nature, 267, 626-630

17. Tannenbaum, J., Splawinsky, J.A., Oates, J.A., and Nies, A.S. (1975) Enhanced Renal Prostaglandin Production in the Dog: 1 Effects on Renal Function. Circulation Research, 36, 197-203

18. Feigen, L.P., Klainer, E., Chapnick, B.M. and Kadowitz, P.J. (1976) The Effect of Indomethacin on Renal Function in Pentobarbital - Anaesthetised Dogs. Journal of Pharmacology and Experimental Therapeutics, 198, 457-463

19. Lonigro, A.J., Itskovitz, H.D., Crowshaw, K. and McGiff, J.C. (1973) Dependency of Renal Blood Flow of Prostaglandin Synthesis in the Dog. Circulation Research, 32, 712-717.

20. Barraclough, M.A. and Mills, I.H. (1965) Effect of Bradykinin on Renal Function. Clinical Science, 28, 69-74

21. Webster, M.E. and Gilmore, J.P. (1964) Influence of Kallidin -10 on Renal Functions. American Journal of Physiology, 206, 714-718

22. Nasjletti, A., colina-Chourio, J. and McGiff, J.C. (1975) Disappearance of Bradykinin in the Renal Circulation of Dogs. Circulation Research, 37, 59-65

23. Davalos, M., Frega, N.S., Saker, B. and Leaf, A. (1978) Effect of Exogenous and Endogenous Angiotensin II in the Isolated Perfused Rat Kidney. American Journal of Physiology, 235, F605-610.

24. McGiff, J.C. and Itskovitz, H.D. (1973) Prostaglandins and the Kidney. Circulation Research, 33, 479-488.

25. Data, J.L., Gerber, J.G., Crump, W.J., Frolich, J.C., Hollifield, J.W. and Nies, A.S. (1978) The Prostaglandin System: A Role in Canine Barroreceptor Control of Renin Release. Circulation Research, 42, 454-458.

26. Olsen, U.B. (1977) The Pharmacology of Bumetanide : A Review. Acta Pharmacology and Toxicology, 41 (Suppl. III), 1-31

27. DeForrest, J.M., Davies, J.O., Freeman, R.H., Seymour, A.A., Rowe, B.P., Williams, G.M. and Davis, T.P. (1980) Effects of Indomethacin and Meclofenamate on Renin Release and Renal Haemodynamic Function During Chronic Sodium Depletion in Dogs. Circulation Research, 47, 99-107

28. Johnston, P.A., Bernard, D.B., Perrin, N.S., Arbeit, L., Lieberthal, W. and Levinsky, N.G. (1981) Control of Rat Renal Vascular Resistance During Alterations in Sodium Balance. Circulation Research, 48, 728-733

29. Muirhead, E.E. (1980) The Antihypertensive Functions of the Kidney. Hypertension, 2. 444-464

30. Margolius, H.S., Geller, R., Pissano, J.J. and Sjoerdsma, A. (1971) Altered Urinary Kallikrein in Human Hypertension. Lancet, 2, 1063-1065.

31. Wennmalm, A. (1978) Influence of Indomethacin on the Systemic and Pulmonary Vascular Resistance in Man. Clinical Science, 54, 141-145.

32. Ylitalo, P., Pitkajarvi, T., Metsa-Ketela, T. and Vapaatalo, H. (1978) The Effect of Inhibition of Prostaglandin Synthesis on Plasma Renin Activity and Blood Pressure in Essential Hypertension. Prostaglandins in Medicine, 1, 479-488.

33. Tyson, J.E.A. (1968) Oral Contraception and Elevated Blood Pressure. American Journal of Obstetrics and Gynaecology, 100, 875-876.

34. Weir, R.J., Briggs, E., Mack, A., Naismith, L., Taylor, L. and Wilson E. (1974) British Medical Journal, 1, 533-535.

35. Fisch, I. R., Freedman, S.H. and Myatt, A.V. (1972) Oral Contraceptives, Pregnancy and Blood Pressure. Journal of the American Medical Association, 222, 1507-1510.

36. Dunn, F.G., Jones, J.V., and Fife, R. (1975) Malignant Hypertension Associated with use of Oral Contraceptives. British Heart Journal, 37, 336-338.

37. Kannel, W.B., Gordon, T. and Schwartz, M.J. (1971) Systolic Versus Diastolic Blood Pressure and Risk of Coronary Heart Disease. The Framingham Study. American Journal of Cardiology, 27, 335-346.

38. Kaplan, N.M., (1978) Cardiovascular Complications of Oral Contraceptives. Annual Review of Medicine, 29, 31-40.

39. Helmer, O.M. and Griffiths, R.S. (1952) The Effect of the Administration of Oestrogens on the Renin Substrate (Hypertensinogen) Content of Rat Plasma. Endocrinology, 51,421-426.

40. Crane, M.G., and Harris, J.J. (1974) Effects of Estrogens and Gestagens on the Renin-Aldosterone System. In:Oral Contraceptives and High Blood Pressure, p. 100, ed. by Fegly, M.J. and Fregly, M.S., Gainesville, Fla, Dolphin.

41. Cain, M.D., Walters, W.A., and Catt, K.J. (1971) Effect of Oral Contraceptive Therapy on the Renin-Angiotensin System. Journal of Clinical Endocrinology and Metabolism, 33, 671-676.

42. Bhoola, K.D., Dorey, G. and Jones, C.W. (1974) The Ontogenic Development and Action of Sex Hormones on Kallikrein and Other Proteases in the Submaxillary Gland. Fogarty International Centre Procedings, 27, 365-373.

43. Senior, J. and Whalley, E.T. (1974) Variation in the Oestrogen-Progesterone Ratio and Its Effects on Kininogen Levels in the Rat. Journal of Reproductive Fertility, 41, 425-433.

44. Roncaglioni, M.C., Minno, G., Rayers, I., Gaetano, G. and Donati, M.B., (1979) Increased Prostacyclin-Like Activity in Vascular Tissues from Rats on Long-Term Treatment with an Oestrogen-Progesterone Combination. Thrombosis Research, 14, 793-797

45. Chang, W., Makao, J., Orimo, H. and Mirota, S. (1980) Stimulation of Prostaglandin Cyclooxygenase and Prostacyclin Synthetase Activities by Estradiol in Rat Aortic Smooth Muscle Cells. Biochimica et Biphysica Acta, 620, 472-482.

46. Katayama, S. and Lee, J.B. (1985) Estradiol Stimulates Rat Renopapillary Prostaglandin E_2 (PGE_2) but not PGF_2 Biosynthesis. Endocinology, 117, 656-661.

47. Schirar, A., Capponi, A. and Catt, K.J. (1980) Regulation of Uterine Angiotensin II Receptors by Oestrogen and Progesterone. Endocinrology, 106, 5-12.

48. Roberts, J.M., Insel, P.A., Goldfien, R.D., and Goldfien, A. (1977) Alpha Adrenoreceptors but not Beta Adrenoreceptors Increase in Rabbit Uterus with Estrogen. Nature, 270, 624-625

49. Nissenson, R., Fluoret, G. and Hechter, O. (1978) Opposing Effects of Estradiol and Progesterone on Oxytocin Receptors in Rabbit Uterus. Proceedings of the National Academy of Science U.S.A., 75, 2044-2048.

50. Boyd, W.N., Burden, R.P. and Aber G.M. (1975) Intrarenal Vascular Changes in Patients Receiving Oestrogen-Containing Compounds. A Clinical, Histological and Angiographic Study. Quarterly Journal of Medicine, 44, 415-431.

51. László, F.A., (1981) Renal Cortical Necrosis. Experimental Induction by Hormones. Contributions to Nephrology, 28, 1-216.

52. Fray, J.C.S., Siwek, L.G., Strull, W.M., Steller, R.N. and Wilson, J.M. (1976) Influence of Dietry Sodium on Renin Activity and Arterial Pressure During Anaesthesia. American Journal of Physiology, 231, 1185-1190.

53. Walker, L.A., Buscemi-Bergin, M. and Gellai, M. (1983) Renal Haemodynamics in Conscious Rats: Effects of Anaesthesia, Surgery and Recovery. American Journal of Physiology, 245, F67-F74.

54. Burger, B.M. Hopkins, T., Tullock, A. and Hollenberg, N.K. (1978) The Role of Angiotensin in the Canine Renal Vascular Response to Barbiturate Anaesthesia. Circulation Research, 38, 196-202.

55. Pettinger, W.A., Tanaka, K., Keston, K., Campbell, W.B. and Brooks, S.N. (1975) Renin Release, an Artifact of Anaesthesia and its Implications in Rats. Proceedings of the Society for Experimental Biology and Medicine, 148, 625-630.

56. Walker, L.A., Buscemi-Bergin, M. and Gellai, M. (1983) Renal Haemodynamics in Conscious Rats: Effects of anaesthesia, Surgery and Recovery. American Journal of Physiology, 245, F67-F74.

57. Popovic V. and Popovic P. (1960) Permanent Cannulation of Aorta and Vena Cava in Rats and Ground Squirrels. Journal of Applied Physiology, 15, 727-728.

58. Hageman, W.E., and Brannan, M.D. (1982) Methods of Arterial Pressure Measurement: Direct and Indirect. In: Hypertension Research, p1-45, Radzialowski, F.M. (ed.), New York, Dekker.

59. Bartrum, R.J., Berkowitz, M. and Hollenberg, N.K. (1974) A Simple Radioactive Microsphere Method for Measuring Regional Flow and Cardiac Output. Investigative Radiology, 9, 126-132.

60. Neutze, J.M., Wyler, F. and Rudolph, A.M. (1968) Use of Radioactive Microspheres to Assess Distribution of Cardiac Output in Rabbits. American Journal of Physiology, 215, 486-495.

61. Buckberg, G.D., Luck, J.C., Payne, D.B., Hoffman, J.I.E., Archie, JP. and Fixler, D.E. (1971) Some Sources of Error in Measuring Regional Blood Flow with Radioactive Microspheres. Journal of Applied Physiology, 31, 598-604.

62. Phibbs, R.H., Wyler, F. and Neutze, J. (1967) Rheology of Microspheres Injected into Circulation of Rabbits. Nature, 216, 1339-1340.

63. Sasaki, Y. and Wagner, N. Jr. (1971) Measurement of the Distribution of Cardiac Output in Unanaesthetised Rats. Journal of Applied Physiology, 30, 879-884.

64. Malik, A.B., Kaplan, J.E. and Saba, T.M. (1976) Reference Sample Method for Cardiac Output and Regional Blood Flow Determinations in the Rat. Journal of Applied Physiology, 40, 472-475.

65. Sapirstein, L.A. (1958) Regional Blood Flow by Fractional Distribution of Indicators. American Journal of Physiology, 193, 161-168.

66. Popovic, V.P., and Kent, K.M. (1964) 120-Day Study of Cardiac Output in Unanaesthetised Rats. American Journal of Physiology, 207, 767-770.

67. Homey, C.J., and Haber, E. (1979) Radionuclide Displacement Assays. In: Cardiovascular Nuclear Medicine, p140 Strauss, H.W., and Pitt, B. (eds.) St. Louis, Mosby.

68. Bing, J. and Poulson, K. (1969) Cause of Increased Plasma Angiotensinogen After Nephrectomy. Acta. Pathology and Microbiology of Scandinavia, 78, 669-673.

69. Haas, E., Goldblatt, H., Gipson, E.C. and Lewis, L. (1966) Extraction, Purification and Assay of Human Renin Free of Angiotensinase. Circulation Research, 19, 739-749

70. Sealey, J.E., Moon, C., Laragh, J.H. and Alderman, M. (1976) Plasma Prorenin: Cryoactivation and Relationship to Renin Substrate in Normal Subjects. American Journal of Medicine, 61, 731-738

71. Rodbard, D., Rayford, P.L., Cooper, J. and Ross, G.T. (1968) Statistical Quality Control of Radioimmunoassay. Journal of Clinical Endocrinology, 28, 1412-1418.

72. Woodhead, J.S., (1980) The Control of Performance in Immunoassays. In: Immunoassays for the 80's, Voller, A., Bartlett, A. and Bidwell, D. (eds.), MTP Press, Lancaster.

73. Lowry, O.H., Rosebrough, N.J., Lewis Farr, A. and Randall, R.J. (1951) Protein Measurement with the Folin Phenol Reagent. Journal of Biological Chemistry, 193, 265-275.

74. Bensadoun A. and Weinstein, D. (1976) Assay of Proteins in the Presence of Interfering Materials. Analytical Biochemistry, 70, 241-250.

75. Peterson, G.L. (1983) Determination of Total Protein. Methods in Enzymology, 91, 95-105.

76. Scroop, G.C. and Lowe, R.D. (1969) Efferent Pathways of the Cardiovascular Response to Vertebral Artery Infusions of Angiotensin in the Dog. Clinical Science, 37, 605-619.

77. Schrier, R.W., McDonald, K.M., Wells, R.E., and Lawler, D.P. (1970) Influence of Hematocrit and Colloid on Whole Blood Viscosity During volume Expansion. American Journal of Physiology, 218, 346-352.

78. Gardner, M.J. and Altman, D.G. (1986) Confidence Intervals Rather than P Values: Estimation Rather than Hypothesis Testing. British Medical Journal, 292, 746-750 .

79. Sokal, R.R. and Rohlf, F.J. (1969) In: Biometry. W.H. Freeman and Company, San Francisco.

80. Glasser, S.R. (1954) Influence on an Adequate Dietary Protein on the Immediate and Latent Effects of Stilbestrol. American Journal of Physiology, 179, 421-428.

81. Dignam, W.S., Voskain, J. and Assali, N.S. (1956) Effects of Estrogens on Renal Haemodynamics and Excretion of Electrolytes in Human Subjects. Journal of Clinical Endocrinology, 16, 1032-1042.

82. Hollenberg, N.K., Williams, G.H., Berger, E., Chenitz, W., Hoosmond, I. and Adams, D.F. (1976) Renal Blood Flow and its Response to Angiotenin II. Circulation Research, 38, 35-40

83. Dean, A.L., Abels, J.C. and Taylor, H.C. (1945) The Effects of Certain Hormones on the Renal Function of Man. Journal of Urology, 53, 647-651.

84. Dance, P., Lloyd, S. and Pickford, M. (1959) The Effects of Stillboestrol on the Renal Activity of Conscious Dogs. Journal of Physiology, 145, 225-240

85. King, R.J.B. and Mainwaring, W.I.P. (1974) In : Kidney in Steroid-Cell Interactions, p247. University Park Press, Baltimore.

86. Gould, A.B., Skegge, L.T. and Kahn, J.R. (1963) The Presence of Renin Activity in Blood Vessel Walls. Journal of Experimental Medicine, 118, 389-399.

87. Thurston, H. and Swales, J.D. (1974) Action of Angiotensin Antagonists and Antiserum Upon the Pressor Response to Renin : Further Evidence for the Local Generation of Angiotensin II. Clinical Science, 46, 273-276.

88. Seeman, P. (1966) Erythrocyte Membrane Stabilisation by Steroids and Alcohols : A Possible Model for Anaesthesia. Biochemical Pharmacology, 15, 1632-1637.

89. Fregly, M.J. (1973) Effect of chronic Administration of an Oral Contraceptive on the Blood Pressure of Rats. Toxicology and Applied Pharmacology, 25, 560-568.

90. Saruta, T., Nakamura, R., Saito, I., Kendo, K. and Matuki, S. (1975) Oestrogen Hypertension in Rats. Clinical Science, 48, 457-460.

91. Thorn, G.W. and Engel, L.L. (1938) The Effect of Sex Hormones on the Renal Excretion of Electrolytes. Journal of Experimental Medicine, 68, 299-312.

92. Nocenti, M.R. and Cizek, L.J. (1964) Influence of Estrogens on Electrolyte and Water Exchanges in the Ovariectomised Rat. American Journal of Physiology, 206, 476-482.

93. Johnson, J.A., Davis, J.O., Baumer, J.S. and Schneider, E.G. (1970) Effects of Estrogens and Progesterone on Electrolyte Balances in Normal Dogs. American Journal of Physiology, 219, 1691-1697.

94. Fluhmann, C.F. (1934) A New Procedure for the Determination of Estrin in the Blood of Women. Endocrinology, 18, 705.

95. Thorn, G.W., Nelson, K.R. and Thorn, D.W. (1938) A study of the Mechanism of Oedema Associated with Menstruation. Endocrinology, 22, 155-163.

96. Hoffmann, J. (1944) In : Female Endocrinology, Chap. 40 p.681, W.B. Saunders Company, Philadelphia.

97. Johnson, J.A., Davis, J.O., Brown, P.R., Wheeler, P.D., and Witty, R.T. (1972) Effects of Estradiol on Sodium and Potassium Balances in Adrenalectomised Dogs. American Journal of Physiology, 223, 194-197.

98. Crocker, A.D. (1970) Variations in Mucosal Sodium and Water Transfer Associated with the Rat Oestrus Cycle. Journal of Physiology, 214, 257-264.

99. Stubbs, D.H., Johnson, J.A., Ichikawa, S., Fowler, W.L. Jr. Kilfoil, J. and Keitzer, W.F. (1980) Role of Angiotensin II in Oral contraceptive Hypertension in Anaesthetised Rats. Life Sciences, 27, 435-440

100. Poulsen, K., Vuust, J. and Lund, T. (1980) Renin Precursor From Mouse Kidney Identified by Cell-Free Translation of Messenger RNA. Clinical Science, 59, 297-299.

101. Rougeon, F., Chambraud, B., Foote, S., Panthier, J.J., Nageoth, R. and Corvol, P. (1981) Molecular Cloning of A Mouse Submaxillary Gland Renin cDNA Fragment. Proceedings of the National Academy of Science of the U.S.A., 78, 6367-6371.

102. Panthier, J.J., Foote, S., Chambraud, B., Strasberg, A.D., Corval, P. and Rougeon, F. (1982) Complete Amino Acid Sequence and Complete Maturation of the Mouse Submaxillary Gland Renin Precursor. Nature, 298, 90-92

103. Misono, K.S., Chang, J.J. and Inagami, T. (1982) Amino Acid Sequence of Mouse Submaxillary Gland Renin. Proceedings of the National Academy of Science of the U.S.A., 79, 4858-4862.

104. Lumbers, E.R. (1971) Activation of Renin in Amniotic Fluid By Low pH. Enzymologica, 40, 329-336.

105. Noth, R.H., Cariski, A.T. and Havelick, J. (1980) Tryptic Activation of Plasma Renin Activity. Journal of Clinical Endocrinology and Metabolism, 50, 983-988.

106. Morris, B.J. (1978). Activation of Human Inactive (Pro-) Renin By Cathepsin D and Pepsin. Journal of Clinical Endocinology and Metabolism, 46, 156-157.

107. Derkx, F.H.M., Bouma, B.N., Schalekamp, M.P.A. and Schalekamp, M.A.D.A. (1979) An Intrinsic Factor XII- Prekallikrein - Dependant Pathway Activates the Human Plasma Renin-Angiotensin System. Nature, 280, 315-318.

108. Sealey, J.E., Atlas, S.A., Laragh, J.H., Oza, N.B. and Ryan, J.W. (1978) Human Urinary Kallikrein Converts Inactive to Active Renin and is a Possible Physiological Activator of Renin. Nature, 275, 144-145.

109. Derkx, F.H.M., Tan-Tjiong, W.L., Man In't Veld, A.J., Schalekamp, M.P.A. and Schalekamp, M.A.D.H. (1979) Activation of Inactive Plasma and Tissue Kallikreins. Clinical Science, 57, 351-357.

110. Sealey, J.E., Moon, C., Laragh, J.H. and Alderman, M. (1976) Plasma Prorenin: Cryoactivation and Relationship to Renin Substrate in Normal Subjects. American Journal of Medicine, 61, 731-738.

111. Leckie, B.J. (1981) Inactive Renin: An Attempt at a Perspective. Clinical Science, 60, 119-130

112. Slater, E.E. and Haber, E. (1979) Inactive renin - "through a glass darkly". New England Journal of medicine, 301, 429-430

113. Derkx, F.H.M., Van Gool, J.M.G., Wenting, G.J., Verhoeven, R.P., Man In't Veld, A.J. and Schalekamp, M.A.D.A. (1976) Inactive renin in human plasma. Lancet ii, 496-498.

114. Grace, S., Munday, K.A., Noble, A.R. and Richards H.K. (1979) Plasma active and inactive renin in the rabbit; effect of dietary sodium depletion and repletion. Journal of Physiology (London), 292, 421-428.

115. Millar, J.A., Leckie, B.J., Semple, P.F., Morton, J.J., Sonkodi, S. and Robertson, J.I.S. (1978) Active and inactive renin in human plasma, renal arteriovenous differences and relationships with angiotensin and renin-substrate. Circulation Research, 43 (Suppl 1), 120-127.

116. Medlin, A.M., Loh, A.Y. and Osmond, D.H. (1979) Fibrinolysis, Renin Activity, and Prorenin in Normal Women: Effects of Exercise and Oral contraceptive Medication. Journal of Clinical Endocrinology and Metabolism, 49, 663-671.

117. Barrett, J.D., Eggena, P. and Sambhi, M.P. (1981) Trypsin Activable Renin in Rat Plasma. In: Heterogeneity of Renin and Renin substrate, pp. 73-82. Ed. Sambhi, M.P., Elsevier North Holland, New York.

118. Glorioso, N., Madeddu, P., Dessi-Fulgheri, P., Giuseppe, F., Maloni, F., Bandiera, F., Tonolo, G. and Rapelli, A. (1983) Trypsin-Activable Inactive Renin in Rat Plasma. Clinical Science, 64, 137-140.

119. Hsueh, W.A., Carlson, E.J. and Dzau, V.J. (1983) Characterisation of Inactive Renin From Human Kidney and Plasma. Journal of Clinical Investigation, 71, 506-517.

120. Sealey, J.E., Atlas, S.A. and Laragh, J.H. (1980) Prorenin and Other Large Molecular Weight Forms of Renin. Endocrine Reviews, 1, 365-391.

121. Osmond, D.A. and Cooper, R.N. (1977) Cryoactivation and Tryptic Activation of Blood 'Prorenin' in Normal Man and Animals. Canadian Journal of Physiology and Pharmacology, 56, 792-805.

122. Vandongen, R., Poussée, N., Strong, K.D. and Berkenhager, W.H. (1977) Evidence that 'Inactive' Renin is Produced Outside the Kidney of the Rat. Clinical Science, 53, 189-191.

123. Lauritzen, M., Damsgaard, J.J., Rubin, I. and Lauritzen, E. (1976) A Comparison of the Properties of Renin Isolated From Pig and Rat Kidney. Biochemical Journal, 155, 317-323.

124. Nakane, H., Nakane, Y., Corval, P. and Menard, J. Effect of Acid, Trypsin and Cold Treatment and of Renin-Plasma Interaction on the activity of renin secreted by rat kidney. Clinical Science, 57, 233-240.

125. Morris, B.J. and Johnston, C.I. (1976) Isolation of Renin Granules from rat kidney cortex and evidence for an inactive form of renin (prorenin) in granules and plasma. Endocrinology, 98, 1466-1474.

126. Inagami, T., Hirose, S., Murakami, K. and Matoba, T. (1977) Native form of renin in the kidney. Journal of Biological Chemistry, 252, 7733-7737.

127. Munday, K.A., Noble, A.R. and Richards, H.K. (1982) Inactive and Active Renin Release from rabbit kidney cortex slices: effect of Sodium concentration and of furosemide. Journal of Physiology, 328, 421-430.

128. Derkx, F.H.M., Van Gool, J.M.G., Wenting, G.J., Verhoeven, R.P., Man In't Veld, A.J. and Schalekamp, M.A.D.H. (1976) Inactive Renin in human plasma. Lancet, ii, 496-499.

129. Takii, Y., Figueiredo, A.F.S. and Inagami, T. (1985) Application of immunochemical methods to the identification and characterisation of rat kidney inactive renin. Hypertension, 7, 236-243.

130. Wong, P.Y., Tolamo, R.C., Williams, G.H. and Colman, R.W. (1975) Response of the Kallikrein-kinin and renin-angiotensin systems to saline infusion and upright posture. Journal of Clinical Investigation, 55, 691-698.

131. Mersey, J.H., Williams, G.H., Hollenberg, N.K., Edmonds, R. and Dhley, R. (1977) Relationship between aldosterone and bradykinin. Circulation Research, Suppl. 1, 84-88.

132. Gross, F., Brunner, A. and Ziegler, M. (1965) Renin-angiotensin system, aldosterone and sodium balance. Recent Progress in Hormonal Research, 21, 119-177.

133. Vander, A.J. (1967) Control of renin release. Physiological Review, 47, 359-382.

134. Davis, J.O. and Freeman, R.H. (1976) Mechanisms regulating renin release. Physiological Reviews, 56, 1-56.

135. Mikache, L.W., Mikache, U. and Gross, F. (1970) Effect of Sodium restriction on renal hypertension and on renin activity in the rat. Circulation Research, 27, 973-984.

136. Pettinger, W.A., Marchelle, M. and Augusto, L. (1971) Renin Supression by DOC and NaCl in the rat. American Journal of Physiology, 221, 1071-1074.

137. Fray, J.C.S., Siwek, L.G., Strull, W.M., Steller, R.N. and Wilson, J.M. (1976) Influence of dietary sodium on renin activity and arterial pressure during anaesthesia. American Journal of Physiology, 231, 1185-1190.

138. Mimran, A., Guiod, L. and Hollenberg, N.K. (1974) The role of angiotensin in the cardiovascular and renal response to salt restriction. Kidney International 5, 348-355.

139. Wong, P.C. and Zimmerman, B.G. (1982) Dependance of Renal Vasodilator Effect of Captopril on prevailing plasma renin level in the dog: influence of DOCA - salt treatment. Clinical Science, 63, 355 - 366.

140. Arendshorst, W.J. and Finn, W.F. (1977) Renal Haemodynamics in the rat before and during inhibition of angiotensin II. American Journal of Physiology, 233, F290-F297.

141. Tobain, L. Salt and Hypertension. In: Hypertension: physiopathology and treatment, 73–83, eds. Genest, J., Kuchel, O., Hamet, P. and Cantin, M., 2nd edition, McGraw Hill, New York.

142. Morgan, T., Adam, W., Gillies, A., Wilson, M., Morgan, G. and Carney, S. (1978) Hypertension treated by salt restriction. Lancet, 1, 227–230.

143. Grobbee, D.E. and Hofman, A. (1986) Does Sodium restriction lower blood pressure. British Medical Journal, 293, 27–29.

144. Slack, B.L. and Ledingham, J.M. (1977) The effect of dietary sodium intake on the blood pressure and cardiac output responses to angiotensin II in unanaesthetised rats. Clinical Science, 52, 571–576.

145. Casellas, D. and Mimran, A. (1980) Measurement of cardiac output and its distribution in rats under various sodium intakes, using 15 and 10 micron spheres. Circulation Research, 14, 577–581.

146. Freeman, R.H., Davis, J.O., Vitale, S.J. and Johnson, J.A. (1973) Intrarenal role of angiotensin II. Homeostatic regulation of renal blood flow in the dog. Circulation Research, 32, 692–698.

147. Mimran, A., Guiod, L. and Hollenberg, N.K. (1974) The role of angiotensin in the cardiovascular and renal response to salt restriction. Kidney International, 5, 348–355.

148. Arendshorst, W.J. and Finn, W.F. (1977) Renal Haemodynamics in the rat before and during inhibition of angiotensin II. American Journal of Physiology, 233, F290–F297.

149. Baer, L., Parra-Carrillo, J.Z., Radichevich, I. and Williams, G.S. (1977) Detection of Renovascular Hypertension with angiotensin II blockade. Annals of Internal Medicine, 86, 257–260.

150. Pals, D.T., Masucci, F.D., Denning, G.S., Sipos, F. and Fessler, D.C. (1971) Role of the pressor action of angiotensin II in experimental hypertension. Circulation Research, 29, 673–681.

151. Bakhle, Y.S. (1971) Inhibition of angiotensin I converting enzyme by venom peptides. British Journal of Pharmacology, 43, 252.

152. Ferreira, S.A. (1965) A bradykinin-potentiating factor (BPF) present in the venom of Bothrops jararaca. British Journal of Pharmacology, 24, 163–169.

153. Marshall, G.R. (1976) Structure-activity relations of antagonists of the renin-angiotensin system. Federal Proceedings, 35, 2494–2501.

154. Mimran, A., Hinrichs, J. and Hollenberg, N.K. (1974) Characterisation of smooth muscle receptors for angiotensin: studies with an antagonist. American Journal of Physiology, 226, 185–190.

155. Hollenberg, N. (1979) Pharmacologic interruption of the renin-angiotensin system. Annual Review of Pharmacology and Toxicology, 19, 559–582.

156. Re, R., Nevelina N., Escourrou, M.T., Athanassoulis, C., Burton, J. and Haber, E. (1978) Inhibition of angiotensin-converting enzyme for diagnosis of renal-artery stenosis. New England Journal of Medicine, 298, 582-586.

157. Williams, G.H., McDonnell, L.M., Raux, M.C. and Hollenberg, N.K. (1974) Evidence for different angiotensin II receptors in rat adrenal glomerulosa and rabbit vascular smooth muscle cells. Studies with competative antagonists. Circulation Research, 34, 384-390.

158. Brunner, H.R., Gavras, H., Laragh, J.H. and Keenan, R. (1973) Angiotensin II blockade in man by Sar^1-Ala^8-angiotensin II for understanding and treatment of high blood pressure. Lancet, 2, 1045-1048.

159. Fowler, W.L., Johnson, J.A., Ichikawa, S. and Kietzer, W.F. (1977) The role of angiotensin II in the maintenance of arterial blood pressure during pregnancy in rats. (abst.) Federal Proceedings, 36, 618.

160. Stubbs, D.H., Johnson, J.A., Ichikawa, S., Fowler, W.L., Kilfoil, J. and Keitzer, W.F. (1980) Role of angiotensin II in oral contraceptive hypertension in anaesthetised rats. Life Sciences, 27, 435-440.

161. Ishikawa, I. (1975) Blockade of the systemic and renal vascular actions of angiotensin II with the 1-Sar, 8-Ala analogue in the rat. Life Sciences, 17, 121-130.

162. Gavras, H., Brunner, H.R., Laragh, J.H., Vaughan, E.D. Jr., Koss, M., Cote, L.J. and Gavras, I. (1975) Malignant hypertension resulting from deoxycorticosterone acetate and salt excess: role of renin and sodium in vascular changes. Circulation Research, 36, 300-309.

163. Ballucci, A. and Wilkes, B.M. (1984) Mechanisms of sodium modulation of glomerular angiotensin receptors in the rat. Journal of Clinical Investigation, 74, 1593-1600.

164. Bing, J. and Poulsen, K. (1975) Time course of changes in plasma renin after blockade of the renin system. Acta Pathology and Microbiology of Scandinavia, 83, 454-466.

165. Weinberger, M.H., Collins, R.D., Dowdy, A.J., Nokes, G.W. and Luetscher, J.A. (1969) Hypertension induced by oral contraceptives containing estrogen and gestagen. Annals of Internal Medicine, 71, 891-902.

166. Newton, M.A., Sealey, J.E., Ledingham, J.G.G., and Laragh, J.H. (1968) High blood pressure and oral contraceptives. American Journal of Obstetrics and Gynaecology, 101, 1037-1045.

167. Smirk, F.H. and Hall, W.H. (1958) Inherited hypertension in rats. Nature, 182, 727-728.

168. Okamoto, K. and Aoki, K. (1963) Development of a strain of spontaneously hypertensive rats. Japanese Circulation Journal, 27, 282-293.

169. Bunag, R.D. (1975) Exaggeration of experimental hypertension in rats by contraceptive steroids (enovid). Endocrinology, 98, 1315-1320.

170. Stier, F.M., Woods, J.W. and Dahl, L.K. (1973) Contraceptive steroids and hypertension: an experimental model. Proceedings of the Society of Experimental Biology in Medicine, 143, 561-564.

171. Lew, G.M. (1978) Effects of mestranol on blood pressure and norepinephrine in young normotensive and genetically hypertensive rats. General Pharmacology, 9, 163-166.

172. Laffan, R.J., Goldberg, M.E., High, J.P., Schaeffer, T.R., Waugh, M.H. and Rubin, B. (1978) Antihypertensive activity in rats of SQ 14, 225, an orally-active inhibitor of angiotension I-converting enzyme. Journal of Pharmacology and Experimental Therapeutics, 204, 281-288.

173. Yamori, Y. (1983) Physiopathology of the various strains of spontaneously hypertensive rats. In: Hypertension: Physiopathology and Treatment, 556-581, 2nd edition, eds: Genest, J., Kuchel, O., Hamet, P. and Cantin, M., McGraw Hill, New York.

174. Saruta, T., Guillermo, A.S. and Kaplan, N.M.(1970) A possible mechanism for hypertension induced by oral contraceptives. Archives of Internal Medicine, 126, 621-626.

175. Thorn, G.W., Nelson, K.R. and Thorn, D.W. (1938) A study of the mechanism of edema associated with menstruation. Endocrinology, 22, 155-163.

176. Taylor, H.C.Jr., Warner, R.C. and Welsh, C.A. (1939) The relationship of the estrogens and other placental hormones to sodium and potassium balance at the end of pregnancy and in the puerperium. American Journal of Obstetrics and Gynecology, 38, 748-777.

177. Lew, G.M. (1982) Age differences in blood pressure and norepinephrine concentration in normotensive rats after higher doses of estrogen. General Pharmacology, 13, 75-78.

178. Hoag, J.M., Willis, L.R. and Weinberger, M.H. (1977) Estrogen attenuation of the development of hypertension in spontaneously hypertensive rats. American Journal of Physiology, 233, H369-H373.

179. Moore, T.J., Williams, G.H., Dluhy, R.G., Bavli, S.Z., Himathongkam, T. and Greenfield, M. (1977) Altered renin-angiotensin-aldosterone relationships in normal renin essential hypertension. Circulation Research, 41, 167-171

180. Brunner, H.R., Laragh, J.H., Baer, L., Newton, M.A., Goodwin, F.T., Krakoff, L.R., Bard, R.H. and Bühler, F.R. (1972) Essential hypertension: renin and aldosterone, heart attack and stroke. New England Journal of Medicine, 286, 441-449.

181. Williams, G.H., Tuck, M.L., Sullivan, J.M., Dluhy, R.G., and Hollenberg, N.K. (1982) Parallel adrenal and renal abnormalities in young patients with established hypertension. American Journal of Medicine, 72, 907-914.

182. Burns, M.J., Stokdal, W.J. and Lombard, J.H. (1984) Reduced vasoconstrictor reserve in spontaneously hypertensive rats subjected to haemorrhagic shock. Circulatory Shock, 14, 25-37.

183. Armstrong, J.M., Boura, A.L.A., Hamberg, M. and Samuelsson, B. (1976) A comparison of the vasodepressor effects of the cyclic endoperoxides PGG_2 and PGH_2 with those of PGD_2 and PGE_2 in hypertensive and normotensive rats. European Journal of Pharmacology,39, 251-258.

184. Simpson, L.L. (1974) The effect of prostaglandin E2 on the arterial blood pressure of normotensive and spontaneously hypertensive rats. British Journal of Pharmacology,51, 559-564.

185. McGiff, J.C., Crowshaw, K., Terragno, N.A. and Lonigro,A.J. (1970) Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. Circulation Research,27 (suppl. I), 121-130.

186. Goodrich, S.M., and Wood, J.E. (1964) Peripheral venous distensibility and velocity of venous blood flow during pregnancy or during oral contraceptive therapy. American Journal of Obstetrics and Gynaecology,90, 740-744.

187. Gerber, J.G. and Nies, A.S. (1979) The haemodynamic effects of prostaglandins in the rat. Evidence for an important species variation in renovascular responses. Circulation Research,44, 406-410.

188. McGiff, J.C. and Quilley, C.P. (1981) The rat with spontaneous genetic hypertension is not a suitable model of human essential hypertension. Circulation Research,48, 455-463.

189. Vane, J.R. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biology,231, 232-235.

190. Curry, S.H. and Brown, E.A. (1982) Preparation and stability of indomethacin solutions. Canadian Journal of Physiological Pharmacology,60, 988-992.

191. Conrad, K.P. and Colpoys, M.C. (1986) Evidence against the hypothesis that prostaglandins are the vasodepressor agents of pregnancy. Journal of Clinical Investigation,77, 236-245.

192. Levy, J.V. (1977) Changes in systolic arterial blood pressure in normal and spontaneously hypertensive rats produced by acute administration of inhibitors of prostaglandin biosynthesis. Prostaglandins,13, 153-159.

193. Malik, K.U. and McGiff, J.C. (1975) Modulation by prostaglandins of adrenergic transmission in the isolated perfused rabbit and rat kidney. Circulation Research,36, 599-609.

194. Linas, S.L. and Dickmann, D. (1982) Mechanism of the decreased renal blood flow in the potassium depleted conscious rat. Kidney International,21, 757-764.

195. Swarts, S.L., Williams, G.H., Hollenberg, N.K., Levine, L., Dluhy, R.G. and Moore, T.J. (1980) Captopril induced changes in prostaglandin production. Journal of Clinical Investigation,65, 1257-1264.

196. Abe, K., Ito, T., Sato, M., Haruyama,T., Sato, K., Omata,K., Hiwatari, M., Sakurai, Y., Imai, Y. and Yoshinaga, K. (1980) role of Prostaglandins in the antihypertensive mechanism of captopril in low renin hypertension. Clinical Science,59, 141s-144s.

197. Galler,M., Backenroth, R., Folkert, V.W. and Schlondorff, D. (1982) Effect of converting enzyme inhibitors on prostaglandin synthesis by isolated glomeruli and aortic strips from rats. Journal of Pharmacology and Experimental Therapeutics,220, 23-28.

198. Jackson, E.K., Branch, R.A. and Oates,J.S. (1982) Participation of prostaglandins in the control of renin release. In: Prostaglandins and the Cardiovascular System, p255-276, edited by Oates, J.A., Raven Press, New York.

199. de Jong, P.E., Donker, A.J.M., van der Wall, E., Erkelens, D.W., van der Hem, G.K. and Doorenbos, H. (1980) Effect of indomethacin in two siblings with a renin dependant hypertension,hyperaldosteronism and hypokalaemia. Nephron,25, 47-52.

200. Jackson, E.K., Oates, J.A. and Branch, R.A. (1981) Indomethacin decreases arterial blood pressure and plasma renin activity in rats with aortic ligation. Circulation Research,49, 180-185.

201. Sealey, J.L. and Sondern, C.W. (1941) Comparative estrogenic potency of diethyl stilbestrol, estrone,estradiol and estriol. II.Uterine and vaginal changes in infantile rats. Endocrinology,29, 356-362.

202. Friedman, F.N. and Schreiber, A.D. (1985) Effect of estradiol and steroid analogues on the clearance of immunoglobulin G-coated erythrocytes. Journal of Clinical Investigation,75, 162-167.

203. Edelman, R. and Hartroft, P.M. (1961) Localisation of Renin in the juxtaglomerular cells of the Rabbit and Dog through the use of the Fluorescent-Antibody Technique. Circulation Research, 9, 1069-1077.

204. Cook, W.F. (1967) The Detection of Renin in Juxtaglomerular cells. Journal of Physiology (London), 194, 73-74.

205. Latta, H. (1973) Ultrastructure of the Glomerulus and the Juxtaglomerular Apparatus. In: Handbook of Physiology: Renal Physiology, edited by Orloff, J. and Berliner, R.W. Washington D.C., American Physiological Society pp 1-29.

206. Goormaghtigh, N. (1945) Facts in Favour of an Endocrine Function of the Renal Arterioles. Journal of Pathological Bacteriology, 57, 392-393.

207. Page, I.H. and Bumpus, F.M. (editors (1974)) In: Angiotensin pp 591. New York, Springer - Verlag.

208. Meyer, P., Baudouin, M., Fermandjian, S., Worcel, M., Morgat, J.L. and Fromageot, P. (1972) Angiotensin Receptors in Smooth Muscle Cells. In: Hypertension, edited by Genest, J. and Koiw, E. Berlin, Springer-Verlag. pp 495-505.

209. Regoli, D., Park, W.K. and Rioux, F. (1974) Pharmacology of Angiotensin. Pharmacological Review, 26, 69-123.

210. Bohr, D.F. (1974) Angiotensin on Vascular Smooth Muscle. In: Angiotensin (Handbook of experimental pharmacology), 37, 424-440, edited by Page, I.H. and Bumpus, F.M., New York, Springer - Verlag.

211. Graer, J.D., Graer, J., Ardaillou, R. and Mimoune, O. (1974) Evidence for Renal Glomerular Receptors for Angiotensin II. Kidney International, 6, 241-246.

212. Bernik, M.B. (1969) Contractile Activity of Human Glomeruli in Culture. Nephron, 6, 1-10.

213. Hornych, H., Beaufils, M. and Richet, G. (1972) The Effect of Exogenous Angiotensin on Superficial and Deep Glomeruli in the Rat Kidney. Kidney International, 2, 336-343.

214. Blair-West, J.R., Coghlan, J.P., Denton, D.A., Funder, J.W., Scoggins, B.A. and Wright, R.D. (1971) Inhibition of Renin Secretion by Systemic and Intrarenal Angiotensin Infusion. American Journal of Physiology, 220, 1309-1315.

215. Mayer, L.G. and Langford, H.G. (1974) Effects of Angiotensin on the Renal Circulation. In: Angiotensin, edited by Page, I.H., Bumpus, F.M. New York, Springer - Verlag pp 455-474.

216. Freeman, R.H., Davies, J.O., Spielman, W.S. and Lohmeier, T. (1975) High Output Heart Failure in the Dog: Systemic and Intrarenal Role of Angiotensin II. American Journal of Physiology, 229, 474-478.

217. Satoh, S. and Zimmerman, B.G. (1975) Effect of Saralasin on Renal Vascular Resistance. American Journal of Physiology, 229, 640-645.

218. Hall, J.E., Guyton, A.C., Trippodo, N.C., Lohmeier, T.E., McCaa, R.E. and Cowley, A.W.Jr. (1977) Intrarenal Control of Electrolyte Excretion by Angiotensin II. American Journal of Physiology, 232, F538-F544.

219. Trippodo, N.C., Hall, J.E., Lohmeier, T.E. and Guyton, A.C. (1977) Intrarenal role for Angiotensin II in Controlling Sodium Excretion During Dehydration in Dogs. Clinical Science, 52, 454-458.

220. Taugner, R., Hackenthal, E., Helmchen, V., Ganten, D., Kugler, P., Marin-Grez, M., Nobiling, R., Unger, T., Lockwald, I. and Keilbach, R. (1982) The Intrarenal Renin-Angiotensin System: an Immunocyto-chemical study on the localisation of renin, Angiotensin, Converting Enzyme and the Angiotensins in the Kidney of Mouse and Rat. Klin. Wochenschr, 60, 1218-1222.

221. Caldwell, P.R.B., Seegal, B.C. and Hsu, K.C. (1976) Angiotensin Converting Enzyme: Vascular Endothelial localisation. Science, 191, 1050-1051.

222. Erdos, E.G. (1977) The Angiotensin I Converting Enzyme. Federal Proceedings, 36, 1760-1765.

TITLE THE EFFECTS OF OESTROGEN ON RENAL AND SYSTEMIC HAEMODYNAMICS IN THE RAT: INFLUENCE OF INTRARENAL VASOACTIVE SUBSTANCES AND PLASMA VOLUME STATUS

AUTHOR John Kenrick Evans

INSTITUTION and DATE University of Keele 1986

DX

87847